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CALCIUM-INDUCED STRUCTURAL CHANGES IN THE  
REGULATORY DOMAIN OF TROPONIN-C BY  
MULTIDIMENSIONAL NMR SPECTROSCOPY

BY



STÉPHANE M. GAGNÉ

A thesis submitted to the Faculty of Graduate Studies and Research in  
partial fulfillment of the requirements for the degree of Master of Science.

DEPARTMENT OF BIOCHEMISTRY

Edmonton, Alberta

Fall 1994



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General ..... 0503  
Agricultural ..... 0505  
Commerce-Business ..... 0508  
Finance ..... 0509  
History ..... 0510  
Labor ..... 0511  
Theory ..... 0358  
Folklore ..... 0366  
Geography ..... 0351  
Gerontology ..... 0578  
History ..... 0578  
General ..... 0578

Ancient ..... 0579  
Medieval ..... 0581  
Modern ..... 0582  
Black ..... 0328  
African ..... 0331  
Asia, Australia and Oceania ..... 0332  
Canadian ..... 0334  
European ..... 0335  
Latin American ..... 0336  
Middle Eastern ..... 0337  
United States ..... 0337  
History of Science ..... 0585  
Law ..... 0398  
Political Science ..... 0615  
General ..... 0616  
International Law and Relations ..... 0617  
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General ..... 0308  
Anatomy ..... 0309  
Biostatistics ..... 0379  
Botany ..... 0329  
Cell ..... 0353  
Ecology ..... 0369  
Entomology ..... 0793  
Genetics ..... 0410  
Limnology ..... 0307  
Microbiology ..... 0317  
Molecular ..... 0416  
Neuroscience ..... 0433  
Oceanography ..... 0821  
Physiology ..... 0778  
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Hospital Management ..... 0758  
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Ophthalmology ..... 0571  
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Pharmacology ..... 0572  
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Analytical ..... 0488  
Biochemistry ..... 0738  
Inorganic ..... 0490  
Nuclear ..... 0491  
Organic ..... 0494  
Pharmaceutical ..... 0495  
Physical ..... 0754  
Polymer ..... 0405  
Radiation ..... 0605  
Mathematics ..... 0986  
Physics ..... 0606

General ..... 0607  
Acoustics ..... 0798  
Astronomy and Astrophysics ..... 0759  
Astrophysics ..... 0609  
Atmospheric Science ..... 0610  
Atomic ..... 0752  
Electronics and Electricity ..... 0611  
Elementary Particles and High Energy ..... 0756  
Fluid and Plasma ..... 0611  
Molecular ..... 0463  
Nuclear ..... 0756  
Optics ..... 0611  
Solid State ..... 0463  
Statistics ..... 0346

#### Applied Sciences

Applied Mechanics ..... 0984  
Computer Science ..... 0984

Engineering ..... 0537  
General ..... 0538  
Aerospace ..... 0539  
Agricultural ..... 0540  
Automotive ..... 0541  
Biomedical ..... 0542  
Chemical ..... 0543  
Civil ..... 0544  
Electronics and Electrical ..... 0348  
Heat and Thermodynamics ..... 0545  
Hydraulic ..... 0546  
Industrial ..... 0547  
Marine ..... 0794  
Materials Science ..... 0548  
Mechanical ..... 0743  
Metallurgy ..... 0551  
Mining ..... 0552  
Nuclear ..... 0549  
Packaging ..... 0765  
Petroleum ..... 0554  
Sanitary and Municipal ..... 0790  
System Science ..... 0428  
Geotechnology ..... 0796  
Operations Research ..... 0795  
Plastics Technology ..... 0994  
Textile Technology ..... 0994

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General ..... 0621  
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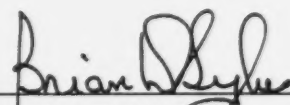
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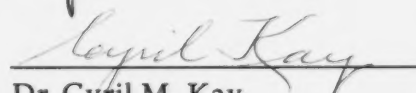
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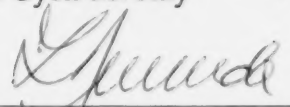
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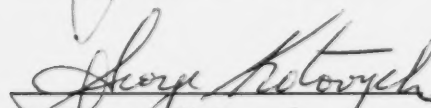
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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Calcium-induced structural changes in the regulatory domain of troponin-C by multidimensional NMR spectroscopy" submitted by Stéphane M. Gagné in partial fulfillment of the requirements for the degree of Master of Science in Biochemistry.

  
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Dr. Zygmunt Derewenda

  
Dr. George Kotovych

  
Dr. Lawrence McIntosh

*Cette thèse est dédiée*

*à la mémoire de mon meilleur ami,*

*Nicolas Zajac*

## ABSTRACT

Multidimensional NMR spectroscopy was used to investigate the calcium-induced structural changes in the regulatory N-domain of troponin-C (1-90). The chemical shift information, along with detailed NOE analysis and  $^3J_{\text{HNH}\alpha}$  coupling constants, permitted the determination and quantification of the  $\text{Ca}^{2+}$ -induced secondary structural change in the N-domain of troponin-C. For both structures five helices and two short  $\beta$ -strands were found, as was observed in the apo N-domain of the crystal structure of whole troponin-C [Herzberg, O. & James, M.N.G. (1988) *J. Mol. Biol.* **203**, 761-779]. The major conformational change observed is the straightening of helix-B upon  $\text{Ca}^{2+}$ -binding. The possible importance and role of this conformational change is explored. Previous circular dichroism studies on the regulatory domain of TnC showed a significant  $\text{Ca}^{2+}$ -induced increase in negative ellipticity, suggesting a significant increase in helical content upon  $\text{Ca}^{2+}$  binding. The present study shows that there is virtually no change in  $\alpha$ -helical content associated with the transition from apo to the  $2\text{Ca}^{2+}$  state of the N-domain of TnC. Therefore the  $\text{Ca}^{2+}$ -induced increase in ellipticity observed by circular dichroism does not relate to a change in helical content, but more likely to changes in spatial orientation of helices. Preliminary tertiary structure calculations of the apo- and calcium-state of the regulatory domain of troponin-C revealed an opening of the structure triggered by the binding of calcium.

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# TABLE OF CONTENTS

|           |                                                                              |           |
|-----------|------------------------------------------------------------------------------|-----------|
| <b>I</b>  | <b>INTRODUCTION</b>                                                          | <b>1</b>  |
|           | <b>From Muscle to Troponin-C: Organization</b>                               | <b>2</b>  |
|           | <i>The organization of striated muscle</i>                                   | 2         |
|           | <i>The thick filament</i>                                                    | 3         |
|           | <i>The thin filament</i>                                                     | 5         |
|           | <i>The troponin complex</i>                                                  | 6         |
|           | <i>Troponin-C</i>                                                            | 7         |
|           | <b>From Ca<sup>2+</sup>-binding to contraction</b>                           | <b>9</b>  |
|           | <i>The sliding-filament mechanism</i>                                        | 9         |
|           | <i>Calcium release and binding to TnC</i>                                    | 10        |
|           | <i>The binding of Ca<sup>2+</sup> induces a conformational change in TnC</i> | 11        |
|           | <i>Ca<sup>2+</sup>-dependant TnC-TnI interactions</i>                        | 11        |
|           | <i>Release of actomyosin ATPase inhibition and power stroke</i>              | 12        |
|           | <b>Thesis Overview</b>                                                       | <b>15</b> |
|           | <b>References</b>                                                            | <b>16</b> |
| <br>      |                                                                              |           |
| <b>II</b> | <b>WHAT WE KNOW</b>                                                          | <b>17</b> |
|           | <b>Characteristics of Troponin-C</b>                                         | <b>18</b> |
|           | <i>The sequence</i>                                                          | 18        |
|           | <i>The binding of Ca<sup>2+</sup></i>                                        | 19        |
|           | <i>The crystal structure</i>                                                 | 20        |
|           | <b>Ca<sup>2+</sup>-induced conformational changes</b>                        | <b>22</b> |
|           | <i>Evidence of conformational changes</i>                                    | 22        |
|           | <i>Model for the Ca<sup>2+</sup>-induced structural change</i>               | 23        |
|           | <b>References</b>                                                            | <b>25</b> |

|            |                                                                                                |           |
|------------|------------------------------------------------------------------------------------------------|-----------|
| <b>III</b> | <b>NMR FOR STRUCTURAL CHARACTERIZATION</b>                                                     | <b>27</b> |
|            | <b>General Theory</b>                                                                          | 28        |
|            | <i>Spins in a magnetic field</i>                                                               | 28        |
|            | <i>From a single spin to many spins</i>                                                        | 30        |
|            | <i>Observation of NMR phenomenon</i>                                                           | 30        |
|            | <i>Chemical shift</i>                                                                          | 33        |
|            | <i>Through-bond correlation</i>                                                                | 34        |
|            | <i>Through-space correlation</i>                                                               | 34        |
|            | <b>From NMR spectra to solution structure</b>                                                  | 38        |
|            | <i>Chemical shift assignment</i>                                                               | 38        |
|            | <i>Secondary structure determination</i>                                                       | 39        |
|            | <i>Tertiary solution structure determination</i>                                               | 40        |
|            | <b>References</b>                                                                              | 42        |
| <b>IV</b>  | <b>GETTING THE DATA</b>                                                                        | <b>43</b> |
|            | <b>First Things First: Getting the Protein</b>                                                 | 44        |
|            | <i>NTnC preparation and <math>^{13}\text{C}</math> and <math>^{15}\text{N}</math> labeling</i> | 44        |
|            | <i>NMR sample preparation</i>                                                                  | 45        |
|            | <b>Data Acquisition</b>                                                                        | 47        |
|            | <i>Overview</i>                                                                                | 47        |
|            | <i>Acquisition and processing</i>                                                              | 47        |
|            | <i>Pulse sequence programming</i>                                                              | 49        |
|            | <b>2D-<math>(^{15}\text{N}, ^1\text{H})</math>-HMQC</b>                                        | 50        |
|            | <b>3D-HNCA</b>                                                                                 | 54        |
|            | <b>3D-HNCOCA</b>                                                                               | 58        |
|            | <b>3D-HNCO</b>                                                                                 | 62        |
|            | <b>3D-HCACO</b>                                                                                | 66        |
|            | <b>3D-<math>^{15}\text{N}</math>-edited-NOESYHMQC</b>                                          | 70        |
|            | <b>3D-<math>^{15}\text{N}</math>-edited-TOCSYHMQC</b>                                          | 73        |
|            | <b>References</b>                                                                              | 76        |

|            |                                                                                      |            |
|------------|--------------------------------------------------------------------------------------|------------|
| <b>V</b>   | <b>BACKBONE ASSIGNMENTS<br/>AND CALCIUM-INDUCED<br/>SECONDARY STRUCTURAL CHANGES</b> | <b>78</b>  |
|            | <b>Backbone assignment</b>                                                           | 80         |
|            | <i>Assignment of NTnC-apo</i>                                                        | 80         |
|            | <i>Assignment of NTnC·2Ca</i>                                                        | 80         |
|            | <b>Secondary Structure</b>                                                           | 85         |
|            | <i>Secondary structure determination strategy</i>                                    | 85         |
|            | <i>NTnC-apo; secondary structure</i>                                                 | 88         |
|            | <i>NTnC·2Ca; secondary structure</i>                                                 | 91         |
|            | <b>Ca<sup>2+</sup>-induced conformational changes</b>                                | 93         |
|            | <i>Secondary structural change</i>                                                   | 93         |
|            | <i>Chemical shift change</i>                                                         | 97         |
|            | <i>Helical content</i>                                                               | 97         |
|            | <b>References</b>                                                                    | 100        |
| <b>VI</b>  | <b>SIDE-CHAIN ASSIGNMENTS AND<br/>PRELIMINARY TERTIARY STRUCTURE</b>                 | <b>101</b> |
|            | <b>Getting Distance Restraints</b>                                                   | 102        |
|            | <i>Side-chain assignments</i>                                                        | 102        |
|            | <i>NOE assignments</i>                                                               | 106        |
|            | <i>NOE calibration</i>                                                               | 106        |
|            | <b>Tertiary Structure</b>                                                            | 108        |
|            | <i>Structure calculation</i>                                                         | 108        |
|            | <i>Structure families</i>                                                            | 108        |
|            | <i>Structure characteristic</i>                                                      | 110        |
|            | <b>References</b>                                                                    | 113        |
| <b>VII</b> | <b>DISCUSSION</b>                                                                    | <b>114</b> |
|            | <b>CONCLUDING REMARKS</b>                                                            | <b>119</b> |

|                                        |            |
|----------------------------------------|------------|
| <b>APPENDIX A: PULSE SEQUENCE CODE</b> | <b>120</b> |
| 1. HNCA                                | 120        |
| 2. HNCOCA                              | 126        |
| 3. HNCO                                | 131        |
| 4. HCACO                               | 136        |
| 5. <sup>15</sup> N-edited-NOESYHMQC    | 141        |
| 6. <sup>15</sup> N-edited-TOCSYHMQC    | 144        |
| 7. <sup>13</sup> C-edited-NOESYHMQC    | 148        |

|                               |            |
|-------------------------------|------------|
| <b>APPENDIX B: ASSIGNMENT</b> | <b>152</b> |
|-------------------------------|------------|

|                                      |            |
|--------------------------------------|------------|
| <b>APPENDIX C: NOE AND STRUCTURE</b> |            |
| <b>CALCULATION PARAMETERS</b>        | <b>160</b> |
| 1. NOE Calibration                   | 160        |
| 2. NOE Restraints                    | 163        |
| 3. Calculation Parameters            | 180        |

## LIST OF TABLES

|                                                                                                                       |     |
|-----------------------------------------------------------------------------------------------------------------------|-----|
| Table II-1. Amino acid content of NTnC.                                                                               | 18  |
| Table II-2. Interhelix angle differences between<br>the N-domain of TnC and other Ca <sup>2+</sup> -binding proteins. | 21  |
| Table V-1. Backbone <sup>1</sup> H and <sup>15</sup> N chemical shifts for NTnC-apo.                                  | 83  |
| Table V-II. Backbone <sup>1</sup> H, <sup>15</sup> N and <sup>13</sup> C chemical shifts for NTnC·2Ca.                | 84  |
| Table V-III. Location of $\alpha$ -helices in the N-domain of Troponin-C.                                             | 93  |
| Table VI-1. Characteristic of the family of structures.                                                               | 109 |

## LIST OF FIGURES

|                                                                                                                       |    |
|-----------------------------------------------------------------------------------------------------------------------|----|
| Figure I-1. Level of organization in striated muscle.                                                                 | 2  |
| Figure I-2. Muscle structure seen at the electron microscopy level.                                                   | 3  |
| Figure I-3. The structure of myosin.                                                                                  | 4  |
| Figure I-4. A space filling representation of<br>all of the atoms in the current model of myosin S1.                  | 4  |
| Figure I-5. A ribbon diagram of the 3-D structure of actin.                                                           | 5  |
| Figure I-6. An actin filament.                                                                                        | 5  |
| Figure I-7. Arrangement of actin, tropomyosin and<br>troponin components in thin filaments of striated muscle.        | 6  |
| Figure I-8. Schematic representation and effects of<br>$\text{Ca}^{2+}$ on interactions among thin filament proteins. | 6  |
| Figure I-9. Ribbon representation of the structure<br>of turkey skeletal muscle TnC.                                  | 7  |
| Figure I-10. The interaction of actin thin filaments<br>and myosin thick filaments in striated muscle.                | 9  |
| Figure I-11. The cyclic process of muscle contraction.                                                                | 10 |
| Figure I-12. Model of the TnI-TnC interaction.                                                                        | 12 |
| Figure I-13. Model for the regulation of contraction in striated muscle.                                              | 13 |
| Figure I-14. Schematic representation summarizing<br>the various steps involved in muscle contraction.                | 14 |
| Figure II-1. Sequence of chicken skeletal troponin-C.                                                                 | 18 |
| Figure II-2. A helix-loop-helix motif can be<br>symbolized by a right hand.                                           | 19 |
| Figure II-3. Stereo view of the crystal structure of<br>turkey skeletal troponin-C.                                   | 20 |

|                                                                                                                                                        |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure II-4.</b> Far-UV CD spectra of recombinant N-domain.                                                                                         | 23 |
| <b>Figure II-5.</b> Diagrammatic representation<br>of the proposed $\text{Ca}^{2+}$ -induced<br>conformational change in the N-terminal domain of TnC. | 24 |
| <b>Figure III-1.</b> The two allowed spin states for a nucleus of spin 1/2.                                                                            | 28 |
| <b>Figure III-2.</b> The energy separation of a spin 1/2 in a magnetic field.                                                                          | 29 |
| <b>Figure III-3.</b> Application of a pulse.                                                                                                           | 30 |
| <b>Figure III-4.</b> The NMR observed signal.                                                                                                          | 31 |
| <b>Figure III-5.</b> Real life FID.                                                                                                                    | 32 |
| <b>Figure III-6.</b> Pulse sequence of the 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC experiment.                                                       | 35 |
| <b>Figure III-7.</b> Two-dimensional $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC<br>acquired using the pulse sequence of figure III-6.                       | 35 |
| <b>Figure III-8.</b> Pulse sequence of the 2D-NOESY experiment.                                                                                        | 36 |
| <b>Figure III-9.</b> Two-dimensional NOESY spectrum<br>obtained from the pulse sequence of figure III-8.                                               | 37 |
| <b>Figure III-10.</b> Distribution of $^1\text{H}$ chemical shifts in proteins.                                                                        | 38 |
| <b>Figure III-11.</b> Survey of expected<br>NOE's in various secondary structures.                                                                     | 39 |
| <b>Figure III-12.</b> Distribution of $\alpha$ -proton chemical shifts.                                                                                | 40 |
| <b>Figure III-13.</b> Distribution of $\alpha$ -carbon chemical shifts.                                                                                | 40 |
| <b>Figure III-14.</b> Schematic diagram of the various steps<br>involved in a typical protein structure determination by NMR.                          | 41 |
| <b>Figure IV-1.</b> Profile of the separation on G25 column.                                                                                           | 46 |
| <b>Figure IV-2:</b> Correlations observed in 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC.                                                                | 50 |
| <b>Figure IV-3:</b> Pulse scheme of the 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC.                                                                     | 52 |
| <b>Figure IV-4.</b> 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC spectrum of NTnC-2Ca.                                                                    | 53 |
| <b>Figure IV-5:</b> Correlations observed in 3D-HNCA.                                                                                                  | 54 |
| <b>Figure IV-6:</b> Pulse scheme of the 3D-HNCA.                                                                                                       | 55 |
| <b>Figure IV-7.</b> Projection of the 64 planes<br>of the HNCA spectrum of NTnC-2Ca.                                                                   | 57 |
| <b>Figure IV-8:</b> Correlations observed in 3D-HNCOCA.                                                                                                | 58 |



|                                                                                                                                                    |    |
|----------------------------------------------------------------------------------------------------------------------------------------------------|----|
| <b>Figure IV-9:</b> Pulse scheme of the 3D-HNCOCA.                                                                                                 | 59 |
| <b>Figure IV-10:</b> Projection of the 64 planes of<br>the HNCOCA spectrum of NTnC·2Ca.                                                            | 61 |
| <b>Figure IV-11:</b> Correlations observed in 3D-HNCO.                                                                                             | 62 |
| <b>Figure IV-12:</b> Pulse scheme of the 3D-HNCO.                                                                                                  | 63 |
| <b>Figure IV-13:</b> Projection of the 64 planes<br>of the HNCO spectrum of NTnC·2Ca.                                                              | 65 |
| <b>Figure IV-14:</b> Correlations observed in 3D-HCACO.                                                                                            | 66 |
| <b>Figure IV-15:</b> Pulse scheme of the 3D-HCACO.                                                                                                 | 67 |
| <b>Figure IV-16:</b> Projection of the 64 planes<br>of the HCACO spectrum of NTnC·2Ca.                                                             | 69 |
| <b>Figure IV-17:</b> Correlations observed in 3D- <sup>15</sup> N-edited NOESYHMQC.                                                                | 70 |
| <b>Figure IV-18:</b> Pulse scheme of the 3D- <sup>15</sup> N-edited NOESYHMQC.                                                                     | 71 |
| <b>Figure IV-19:</b> Projection of the 64 planes of<br>the <sup>15</sup> N-edited NOESYHMQC spectrum of NTnC·2Ca.                                  | 72 |
| <b>Figure IV-20:</b> Correlations observed in 3D- <sup>15</sup> N-edited TOCSYHMQC.                                                                | 73 |
| <b>Figure IV-21:</b> Pulse scheme of the 3D- <sup>15</sup> N-edited TOCSYHMQC.                                                                     | 74 |
| <b>Figure IV-22:</b> Projection of the 64 planes of<br>the <sup>15</sup> N-edited TOCSYHMQC spectrum of NTnC·2Ca.                                  | 75 |
| <b>Figure V-1:</b> Does Ca <sup>2+</sup> induce any changes<br>in NTnC? Well... a picture is worth a thousand words...                             | 78 |
| <b>Figure V-2:</b> Connectivities related to one residue<br>of NTnC·2Ca, M28, in various 3D-experiments.                                           | 82 |
| <b>Figure V-3:</b> $\phi/\psi$ map showing the relation between<br>the $d_{N\alpha}/d_{\alpha N}$ ratio and the major secondary structure regions. | 86 |
| <b>Figure V-4:</b> Schematic representation of<br>the secondary structure determination of NTnC-apo.                                               | 90 |
| <b>Figure V-5:</b> Schematic representation of<br>the secondary structure determination of NTnC·2Ca.                                               | 92 |
| <b>Figure V-6:</b> Comparison of the NOE connectivities of G43's amide<br>proton for NTnC-apo and NTnC·2Ca in their <sup>15</sup> N-edited NOESY.  | 95 |

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|                                                                                                                                            |     |
|--------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <b>Figure V-7.</b> Comparison of the $^3J_{\text{HNH}\alpha}$ coupling constant of E41 for NTnC-apo and NTnC-2Ca in their HMQC-J spectrum. | 96  |
| <b>Figure V-8.</b> Chemical shift change along the sequence of NTnC induced by the binding of calcium.                                     | 98  |
| <b>Figure VI-1.</b> 3D-NMR experiments used for the side-chain assignments of NTnC-2Ca.                                                    | 103 |
| <b>Figure VI-2.</b> Correlations found for Val83 in the HCCHCOSY spectrum.                                                                 | 104 |
| <b>Figure VI-3.</b> Correlations found for Val83 in the $^{13}\text{C}$ -edited NOESYHMQC spectrum.                                        | 105 |
| <b>Figure VI-4.</b> Solution NMR structure of the regulatory domain of TnC.                                                                | 111 |
| <b>Figure VI-5.</b> Superimposition of the apo and $\text{Ca}^{2+}$ -saturated average NMR structure of the N-domain of TnC.               | 112 |
| <b>Figure VII-1.</b> Schematic representation of the $\text{Ca}^{2+}$ -induced straightening of helix-B.                                   | 116 |

CHAPTER *I*

## INTRODUCTION

*Coordinate movement is essential to life. Whether it is swimming, flying, crawling, or walking, all animals use the same basic mechanism for motor control, the contraction of muscle. Even the injection of venom by a snake involves muscle contraction. A large number of protein structural changes and protein-protein interactions are involved in the process of muscle contraction. Regulation of this process in skeletal and cardiac muscle occurs through the binding of calcium to one of these proteins, troponin-C. This thesis reports the  $\text{Ca}^{2+}$ -induced structural changes which occur in troponin-C that lead to muscle contraction.*

## From Muscle to Troponin-C: Organization

### *The organization of striated muscle*

Figure I-1 shows a typical vertebrate striated muscle at successive levels of organization. Striated skeletal muscles are composed of multinucleated cylindrical fibers, or myofibers, 10 to 100  $\mu\text{m}$  in diameter and up to several centimeters long. Each myofiber contains a bundle of myofibrils, each about 1  $\mu\text{m}$  in diameter. A myofibril is made up of a long chain of contracting units called sarcomeres, which repeat every 2.3  $\mu\text{m}$  along the fibril axis. The periodic structure of the myofibril can be clearly observed by electron microscopy (figure I-2). The electron micrograph shows two bands, the A band (dark) and the I band (light), which alternate. A sarcomere is limited by the Z-lines, located in the center of the I band. The

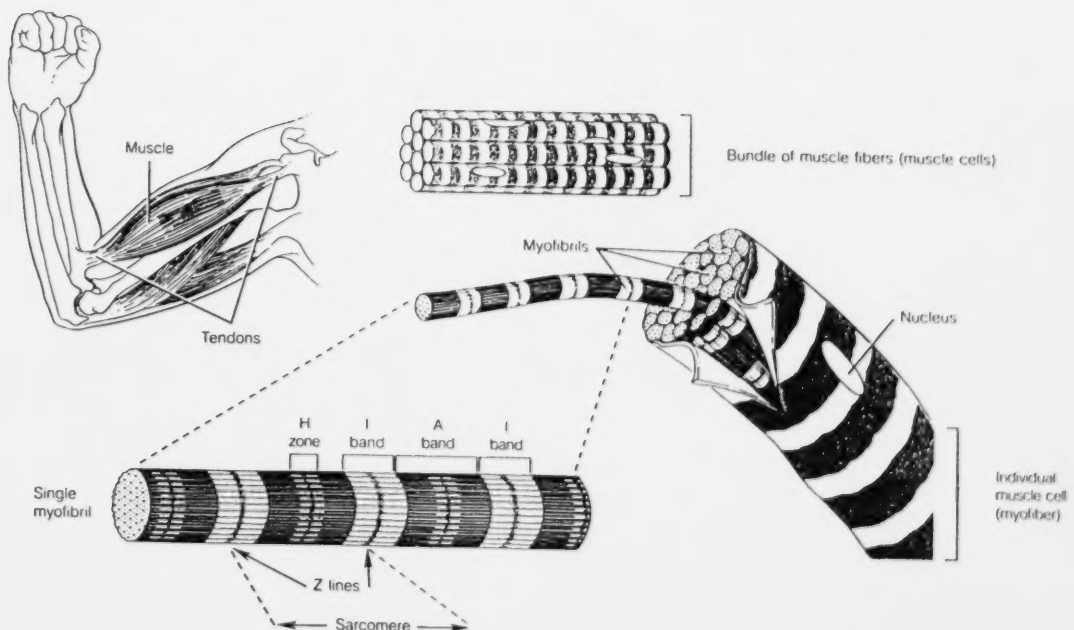


**Figure I-1.** Level of organization in striated muscle.

striations of the myofibrils are the result of periodic variations in concentration of two kinds of interacting filaments, the thick filament and the thin filament. Thick filaments are about  $1.5\ \mu\text{m}$  long and  $150\ \text{\AA}$  wide and are separated by  $400\ \text{\AA}$ . Thin filaments are about  $1\ \mu\text{m}$  long and  $100\ \text{\AA}$  in diameter. In vertebrate muscle each thick filament is surrounded by six thin filaments, and each thin filament lies symmetrically among three thick filaments (figure I-2c). As a consequence of this geometry, there are twice as many thin filaments as thick filaments.

#### *The thick filament*

The major constituent of the thick filament is myosin, a very large protein molecule ( $\approx 520\ \text{kDa}$ ) that looks like a two-headed snake (figure I-3a). Myosin is made of six polypeptide chains: two  $220\ \text{kDa}$  heavy chains and two pairs of light chains (each approximately  $20\ \text{kDa}$ ). The two heavy chains associate to form a two stranded  $\alpha$ -helical coiled coil ( $\approx 1300\ \text{\AA}$  long) with two globular "head" regions at one extremity. Each head is complexed with

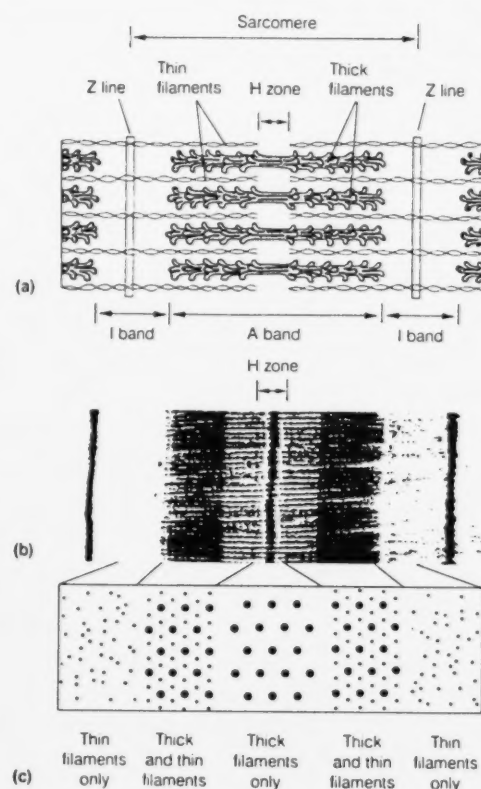


**Figure I-2.** Muscle structure seen at the electron microscopy level. (a) A model of the sarcomere, the repeating unit in striated muscle. The I bands, A bands, and Z lines shown in figure I-1 are identified and structural elements of the sarcomere are indicated. (b) An electron micrograph, showing the same features. (c) A schematic drawing of cross sections of a sarcomere, in the various regions shown in parts (a) and (b). Thick fibers are indicated by heavy dots, thin fibers by small dots.

two different light chains (often referred to as the regulatory and the essential light chains). The coiled coil accounts for the self-association of myosin and the formation of the thick filament backbone (figure I-3b). Each head is complexed with two light chains. The heads of myosin may be enzymatically cleaved by papain to isolate what are called S1 fragments, consisting of the globular regions carrying the two light chains ( $\approx 130$  kDa). The crystal structure of myosin S1 has recently been solved [1], revealing a highly asymmetric molecule with a length of  $165 \text{ \AA}$ , a width of  $65 \text{ \AA}$ , and a thickness of approximately  $40 \text{ \AA}$  (figure I-4).

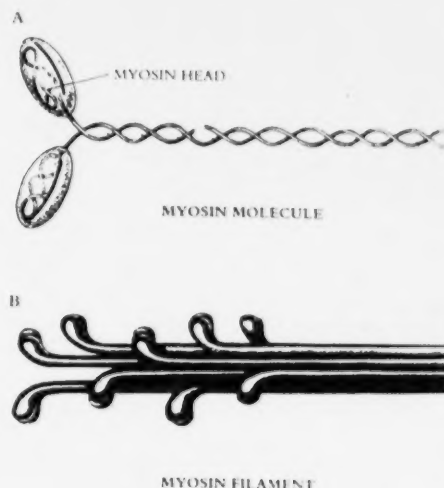


**Figure I-3.** The structure of myosin. (A) Each myosin molecule is a coil of two chains wrapped around one another; at the end of each chain is a globular region referred to as the "head". (B) Myosin molecules are usually combined into filaments, which are cables of myosin from which the heads protrude at regular intervals.

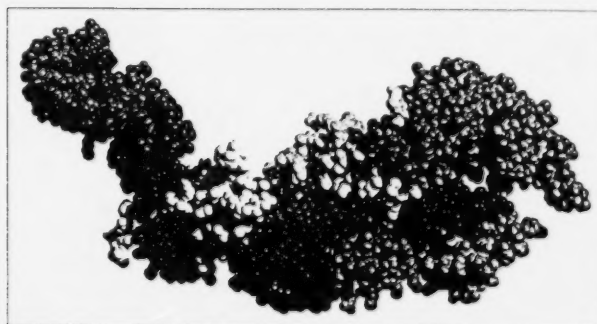


**Figure I-4.** A space filling representation of all of the atoms in the current model of myosin S1. The model is oriented such that the actin binding surface is located at the lower right-hand corner.



*The thin filament*

The thin filament is a more complex structure containing several proteins of which actin is the major building block. The globular monomeric form of actin (G-actin) is a protein of 385 residues arranged into four domains surrounding a deep cleft (figure I-5) [2]. Under physiological conditions, actin polymerizes to form a long, helical fiber (F-actin). The helical stacking of the globular

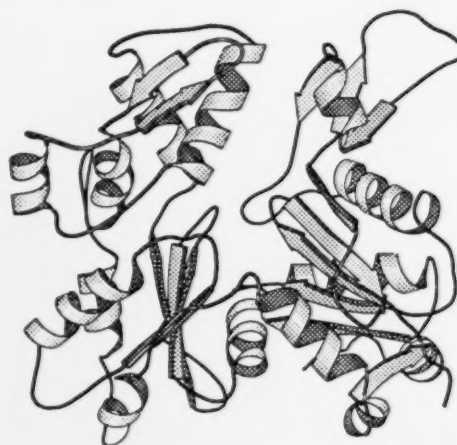


**Figure I-5.** A ribbon diagram of the 3-D structure of actin viewed roughly perpendicular to the flat face of the molecule. The molecule is thought to have a similar orientation in a filament with its long axis vertical. The right side of the molecule is thought to be exposed on the outer surface of the filament.



**Figure I-6.** An actin filament.

monomers gives F-actin the appearance of two strings of beads wound around one another (figure I-6). Although actin is the main constituent of the thin filament, other proteins are essential to the contractile function of striated muscle. One of these is tropomyosin (Tm) which exists as a dimer of two polypeptide subunits ( $\approx 33$  kDa each) that associate in a coiled-coil fashion. Electron micrographs and X-ray diffraction have localized Tm along the actin fiber. Elongated Tm polymerizes by a head-to-tail overlap of 8-11 amino acid residues, such that one Tm molecule spans seven actin monomers. At about one third of the distance from the



carboxy-terminal end of each Tm molecule resides a troponin complex. This troponin complex is formed of three subunits: troponin-T (TnT), troponin-I (TnI), and troponin-C (TnC). The proposed arrangement of actin, Tm, and the troponins in the thin filament is shown in figure I-7.

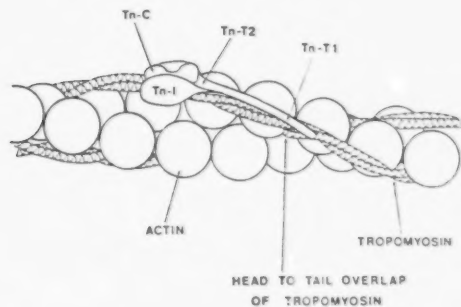


Figure I-7. Arrangement of actin, tropomyosin and troponin components in thin filaments of striated muscle.

### *The troponin complex*

TnT is the largest of the troponin subunits and consists of 259 amino acid residues ( $\approx 30$  kDa). TnT has direct contact with Tm, TnI and TnC, and therefore links the troponin complex to Tm. Electron micrographs have revealed the troponin complex as globular with a long rod-shaped tail identified as TnT [5]. The asymmetric TnT component, represented by its fragments TnT1 (1-158) and TnT2 (159-259), interacts with Tm both at the head-to-tail overlap (TnT1) and in a region about 13-15 nm from its

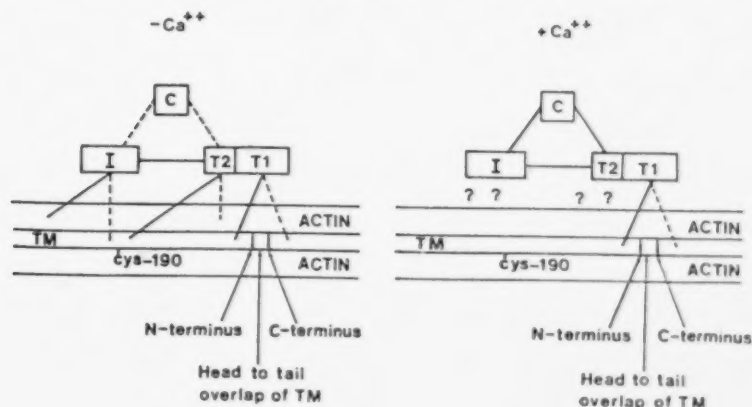


Figure I-8. Schematic representation and effects of  $\text{Ca}^{2+}$  on interactions among thin filament proteins.

C-terminal end (TnT2) (figure I-8) [3, 4]. The second troponin subunit, TnI, has a molecular weight of  $\approx 21$  kDa and binds to the N-terminal end of TnT2 (figure I-8) [6]. In the relaxed state, TnI also interacts with actin and probably with Tm. No tertiary structure of TnI has yet been determined. The third troponin subunit, TnC, is an acidic protein containing 150-163 residues (depending on the source). The interaction between TnC and TnT is uncertain, but some studies show that TnC may interact with the C-terminal end of TnT2 [6]. The interactions between TnC and TnI are, however, well characterized [9], and are found to be  $\text{Ca}^{2+}$ -dependant. A schematic representation of the interactions amongst thin filament proteins is shown in figure I-8.

### *Troponin-C*

TnC ( $\approx 18$  kDa) has four  $\text{Ca}^{2+}$ -binding sites; two high-affinity sites (III and IV) which are believed to be always occupied by either  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  under physiological conditions, and two low-affinity  $\text{Ca}^{2+}$ -specific sites (I and II) which regulate muscle contraction. The crystal structures of turkey skeletal TnC [7] and chicken skeletal TnC [8] reveal a 66%  $\alpha$ -helical protein having two globular domains, each containing two calcium binding sites, connected by an extended  $\alpha$ -helix (figure I-9). In these structures only sites III and IV in the C-terminal domain are occupied by  $\text{Ca}^{2+}$ , whereas the regulatory sites in the N-domain are in the apo state. All four  $\text{Ca}^{2+}$ -binding sites show the helix-loop-helix

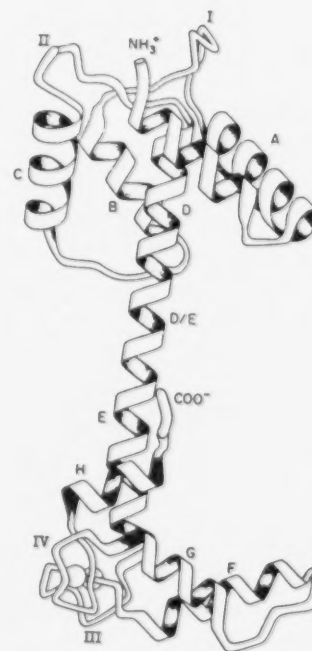


**Figure I-9.** Ribbon representation of the structure of turkey skeletal muscle TnC. Helices are labelled A through H;  $\text{Ca}^{2+}$ -binding sites are labelled I through IV. Sites III and IV are filled with  $\text{Ca}^{2+}$ , whereas sites I and II are apo.

motif termed the EF-hand [10]. The helix packing is, however, different in the  $\text{Ca}^{2+}$ -free regulatory N-domain compared to structures of homologous  $\text{Ca}^{2+}$ -binding proteins which are  $\text{Ca}^{2+}$ -filled [11]. A model for the structure of  $\text{Ca}^{2+}$ -filled N-domain has been proposed based on the homology between the N-domain and the C-domain [14]. The NMR solution structure of the TR<sub>1</sub>C fragment of TnC in the apo-form [12] also shows the same structural features as is found in the N-domain of the crystal structure.

## From $\text{Ca}^{2+}$ -binding to contraction

### *The sliding-filament mechanism*

The sliding-filament mechanism of muscle contraction postulates that the thin filament is displaced relative to the thick filament. For sliding to occur, the S1 portion of myosin attaches to a binding-site on actin to form the actomyosin complex. A conformational change then pulls it toward the center of the sarcomere (figure I-10). S1 has ATPase activity, and it is the hydrolysis of ATP by S1 that provides the free energy necessary to drive muscle contraction. A schematic representation of the proposed mechanism of force production is shown in figure I-11. For the power stroke to occur, myosin must first bind to actin, an event which is regulated by calcium.

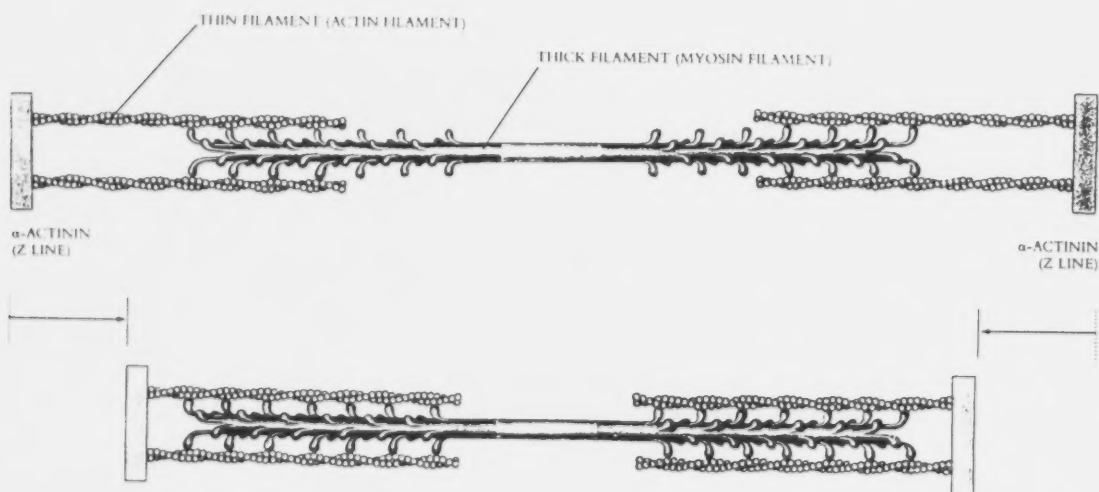


**Figure I-10.** The interaction of actin thin filaments and myosin thick filaments in striated muscle. The heads on the two ends of the myosin filament are oriented in opposite directions, so that as the two ends "walk" along the actin filaments in opposite direction, pulling the actin filaments and there attached  $\alpha$ -actinin (Z-lines) toward the center of the sarcomere. The result is contraction.

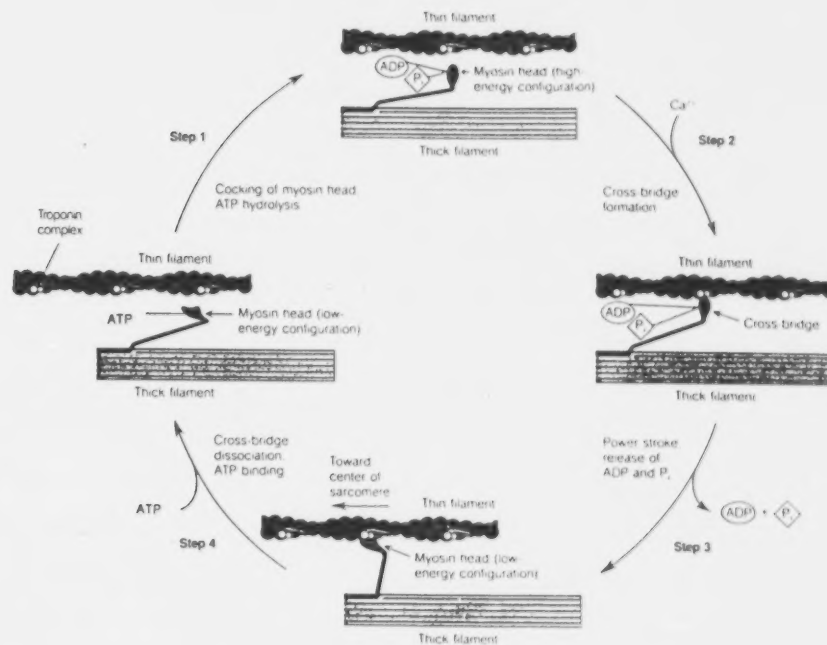


**Figure I-11.** The cyclic process of muscle contraction. Stages in the interaction of one myosin headpiece with a thin filament are shown.

#### *Calcium release and binding to TnC.*

In resting muscle,  $\text{Ca}^{2+}$  ions are actively pumped out of muscle fibers, concentrating the  $\text{Ca}^{2+}$  ions within the spaces of the sarcoplasmic reticulum. The concentration of free  $\text{Ca}^{2+}$  in muscle fiber in the resting state is in the neighborhood of  $10^{-7}$  M, and the concentration of  $\text{Mg}^{2+}$  is about  $10^{-3}$  M, so that the high-affinity sites III and IV of TnC are filled by  $\text{Mg}^{2+}$  ions, and the two low-affinity sites I and II are in the apo state. The crystal structure shown in figure I-9 is therefore related to the conformation of TnC in relaxed muscle. Upon neural stimulation,  $\text{Ca}^{2+}$  is released from the sarcoplasmic reticulum to the cytoplasm (sarcoplasm) of the muscle fiber. This  $\text{Ca}^{2+}$  influx ( $[\text{Ca}^{2+}] > 10^{-6}$  M) causes the binding of two  $\text{Ca}^{2+}$  ions to the

regulatory domain of TnC, inducing a conformational change, and triggering a sequence of events that ultimately lead to muscle contraction.

*The binding of  $\text{Ca}^{2+}$  induces a conformational change in TnC*

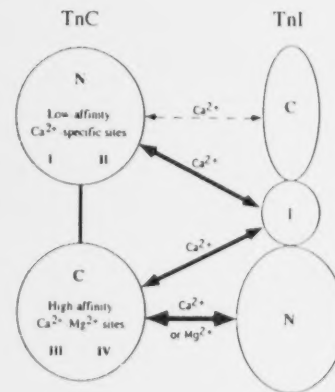
While no structure for the  $\text{Ca}^{2+}$ -saturated state of TnC is known, the  $\text{Ca}^{2+}$ -induced conformational change has been studied extensively using various spectroscopic techniques including NMR, CD, fluorescence and Raman spectroscopy (reviewed in chapter II). These studies have indicated that the C-domain of TnC is mostly unstructured in the apo state, and that the binding of  $\text{Ca}^{2+}$  to site III and IV induces folding. Although previous estimates of the magnitude of the  $\text{Ca}^{2+}$ -induced changes attributable to the N-domain transition are not as clear, a recent CD study has shown significant  $\text{Ca}^{2+}$ -induced ellipticity changes in the isolated recombinant N-domain of TnC [13]. That these changes are a reflection of significant secondary structural alterations was considered a possibility. Although this conformational change has not been fully characterized, it is clear that the conformational change in TnC affects the TnC-TnI interaction.

*$\text{Ca}^{2+}$ -dependant TnC-TnI interactions*

TnI (182 residues) is composed of three regions: N-domain (1-98), C-domain (120-182), and inhibitory region (99-119). The inhibitory region is the portion of TnI required to inhibit the actomyosin  $\text{Mg}^{2+}$ -ATPase (see below). Recent studies on these regions, in conjunction with TnC mutants, have shown a relationship between different TnI regions and TnC domains [9]. Binding studies have revealed that the N-terminal region of TnI interacts with the C-domain of TnC in the relaxed state, and that the inhibitory plus C-terminal region of TnI interacts with the N-domain of TnC in a  $\text{Ca}^{2+}$  dependent manner [9]. These results are summarized in figure I-12. This model suggests that TnC and TnI are always anchored

strongly in an antiparallel fashion via the N-terminal region of TnI and the C-domain of TnC. Only upon the  $\text{Ca}^{2+}$ -induced structural change in the N-domain of TnC does it interact with the C-domain of TnI.

**Figure I-12.** Model of the TnI-TnC interaction. N and C represent the N-terminal and C-terminal domains, respectively, of TnI and TnC. I represents the inhibitory region of TnI. Some of the interactions are dependent on the presence of  $\text{Ca}^{2+}$  and occur only when sites I and II of TnC are filled during muscle activation. The  $\text{Ca}^{2+}$ -dependent interactions occur between the inhibitory region of TnI and both N- and C-terminal domains of TnC. The interactions between the N-terminal region of TnI and the C-terminal domain of TnC are  $\text{Ca}^{2+}$  /  $\text{Mg}^{2+}$ -dependent, and are believed to be present in both the relaxed and the activated state of muscle.



#### *Release of actomyosin ATPase inhibition and power stroke*

The primary role of TnI is to inhibit the formation of the actomyosin complex. When muscle is relaxed, TnI interacts with actin and physically prevents myosin from binding actin. As the N-domain of TnC binds calcium and the interaction between TnC and TnI changes, the interaction between TnI and actin weakens. The troponin-tropomyosin complex is believed to be relocated following  $\text{Ca}^{2+}$ -induced structural change in TnC, allowing the formation of the actomyosin complex (figure I-13). The power-stroke as described in figure I-10 can then occur. The various steps involved in muscle contraction are summarized in figure I-14.



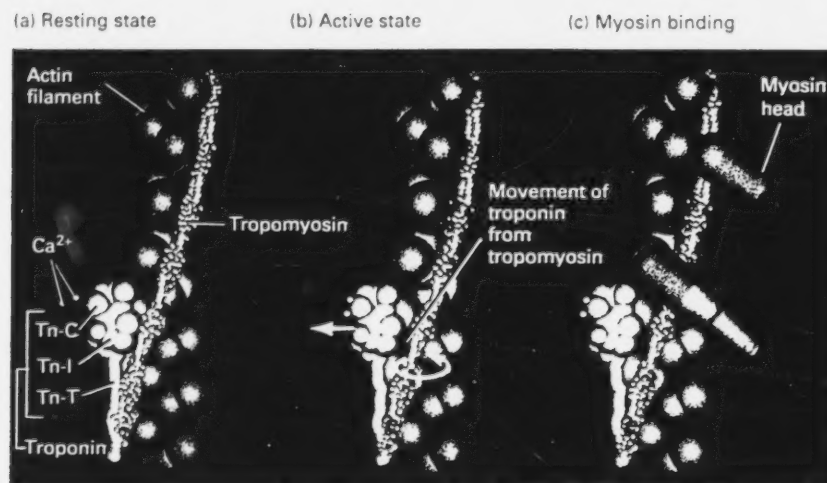


**Figure I-13.** Model for the regulation of contraction in striated muscle. (a) Relaxed muscle. At low  $\text{Ca}^{2+}$  concentrations ( $< 10^{-7}$  M), the configuration of actin, tropomyosin and troponin in the thin filament is such that most myosin headpieces are blocked from contact with the thin fiber. (b) Active state. At higher  $\text{Ca}^{2+}$  concentrations ( $> 10^{-6}$  M), the binding of calcium to TnC causes a rearrangement of the thin-filament fiber components so that myosin binding sites on actin are made available. (c) Myosin binds to actin and the cycle shown in figure I-11 can then occur, leading to muscle contraction.

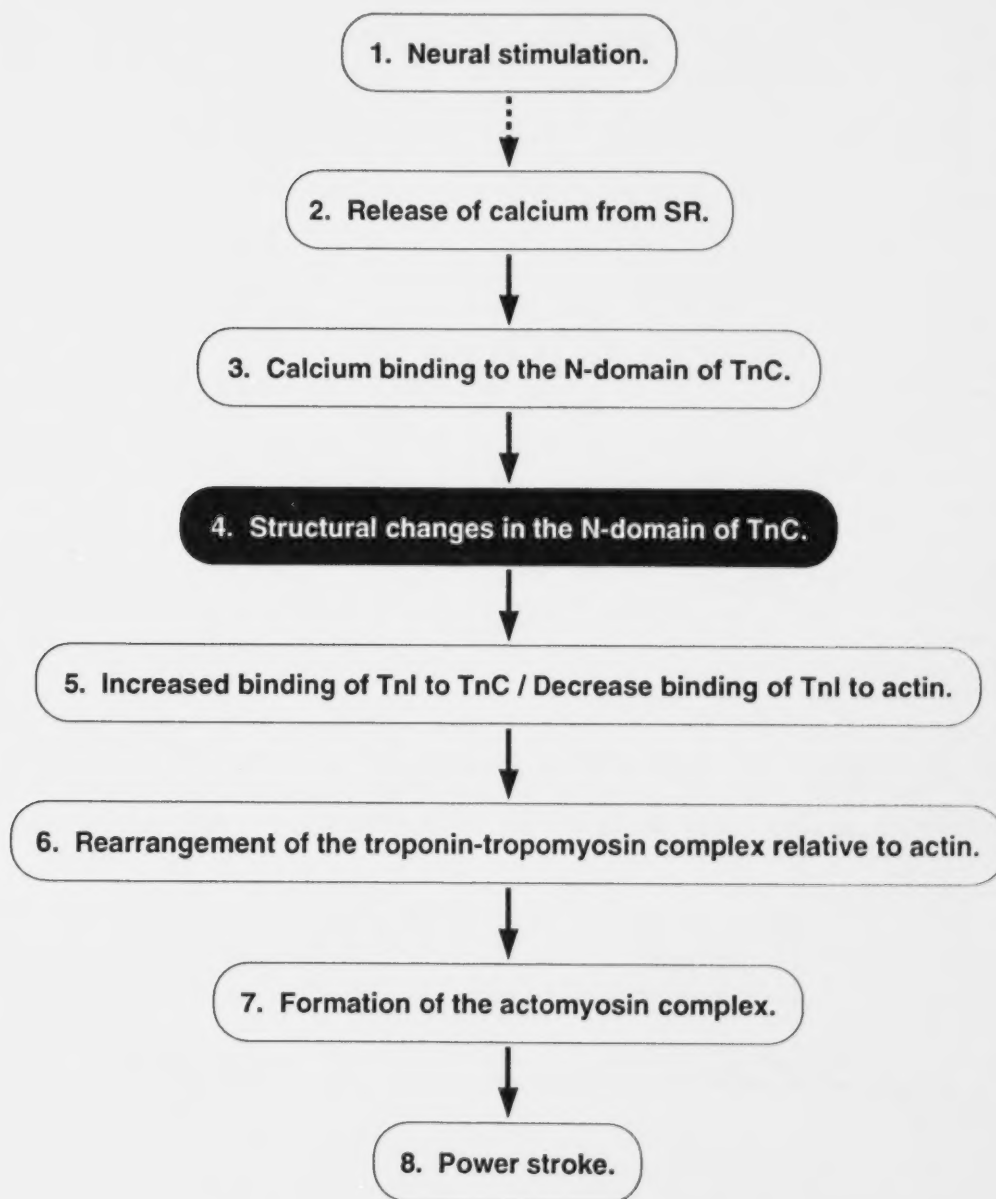


**Figure I-14.** Schematic representation summarizing the various steps involved in muscle contraction. The characterisation of the  $\text{Ca}^{2+}$ -induced structural changes in the regulatory domain of TnC (step 4) is the theme of this thesis.

## Thesis Overview

This thesis reports the nuclear magnetic resonance (NMR) solution structure of the regulatory domain of troponin-C in the  $\text{Ca}^{2+}$ -bound state and the characterization of the  $\text{Ca}^{2+}$ -induced structural changes (step 4 in figure I-15). A large amount of knowledge about TnC has already been acquired, and some of this useful information is outlined in chapter II. Basic NMR theory necessary for the understanding of the interpretation of protein NMR data will be described in chapter III. This work is based mainly on methodologies that were developed concurrently with my research work, and chapter IV therefore describes these state-of-the-art techniques and their implementation. The first large step in the analysis of protein NMR data is the chemical shift assignment of the backbone. Chapter V first describes the strategy used to accomplish this crucial step, and presents the complete backbone assignment. This chemical shift information, along with detailed backbone NOE analysis, permitted the determination of the secondary structure of NTnC in the  $\text{Ca}^{2+}$ -state which is presented in chapter V. This chapter also offers a detailed comparison with the NMR structure of apo-NTnC, the crystal structure of TnC (where the N-domain is apo), and the  $4\text{Ca}^{2+}$  TnC model. Preliminary work on the three-dimensional solution structure of  $\text{Ca}^{2+}$ -bound NTnC is briefly outlined in chapter VI. Finally, chapter VII discusses in detail the  $\text{Ca}^{2+}$ -induced structural changes in the regulatory domain of troponin-C.

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CHAPTER **II****WHAT WE KNOW**

*In 1963, Ebashi discovered a new protein factor that was necessary for the  $\text{Ca}^{2+}$  sensitivity of actomyosin [1,2]. This protein system was called "native tropomyosin", but it was soon found that this system was a complex of tropomyosin and a new globular protein, which was named troponin [3]. Hartshorne and Mueller [4] were the first to observe that troponin was made of subunits. They obtained two fractions, which they named troponin A and troponin B. Troponin A, which also carried the name "EGTA sensitizing factor", was found to be required for calcium control of ATP hydrolysis by myosin [5,6]. Greaser and Gergely [7,8] later resolved troponin into the three subunits we know today and named them troponin-T, troponin-I and troponin-C, the latter corresponding to the troponin A observed previously. In 1972, Murray and Kay [15] reported for the first time that  $\text{Ca}^{2+}$  was inducing a conformational change in troponin A (troponin-C). Up to 1970, there are less than 100<sup>§</sup> publications related to the troponins. In the next decade, about 300 papers characterized troponin-C. Since 1980, approximately 1000 publications have reported data related to TnC. This chapter summarizes some of this data relevant to the course of my research.*

<sup>§</sup> The number of publication is based on a search on the Medline SilverPlatter system.

## Characteristics of troponin-C

### *The sequence*

31 sequences of TnC have been determined so far, going from human skeletal muscle TnC to sea squirt smooth muscle TnC. The sequence of the protein used in the present study, chicken skeletal TnC, is listed in figure II-1. The work presented here relates to the N-terminal domain of TnC (NTnC; residue 1-90;  $M_w = 9977.47$ ), and the amino acid composition of NTnC is listed in table II-1. NTnC contains exactly 50 % hydrophobic residues (hydrophobic amino acids are A, C, F, G, P, I, L, M, V, W, Y) and 50 % hydrophilic residues (hydrophilic amino acids are D, E, K, N, H, Q, R, S, T). NTnC is an acidic protein, with 23 acidic and only 7 basic amino acids.

| Amino Acid | Freq (total) | Freq (percent) |
|------------|--------------|----------------|
| A          | 10           | 11.11          |
| C          | 0            | 0.00           |
| D          | 10           | 11.11          |
| E          | 13           | 14.44          |
| F          | 6            | 6.67           |
| G          | 7            | 7.78           |
| H          | 0            | 0.00           |
| I          | 5            | 5.56           |
| K          | 4            | 4.44           |
| L          | 5            | 5.56           |
| M          | 8            | 8.89           |
| N          | 1            | 1.11           |
| P          | 1            | 1.11           |
| Q          | 4            | 4.44           |
| R          | 3            | 3.33           |
| S          | 4            | 4.44           |
| T          | 5            | 5.56           |
| V          | 4            | 4.44           |
| W          | 0            | 0.00           |
| Y          | 0            | 0.00           |

Table II-1. Amino acid content of NTnC.

1 ASMTDQQA EA RAFLSEEMIA EFKA AFDMFD ADGGGDISTK ELGTVMRMLG  
 51 QNPTKEELDA IIEEVDEDGS GTIDFEEFLV MMVRQMKEDA KGKSEEEELAN  
 101 CFRIFDKNAD GFIDIEELGE ILRATGEHVT EEDIEDLMKD SDKNNDGRID  
 151 FDEFLKMMEG VQ

Figure II-1. Sequence of chicken skeletal troponin-C. The first 90 residues corresponding to the domain used in this study are shown in bold letters.

### The binding of $\text{Ca}^{2+}$

TnC belongs to a family of proteins that use a common motif to bind calcium, the helix-loop-helix or EF-hand motif that was first described by Kretsinger and Nockolds [9]. The spatial arrangement of a typical helix-loop-helix and the correlation with a right hand are shown in figure II-2. TnC possesses 4 of these helix-loop-helix  $\text{Ca}^{2+}$ -binding motifs. Sites III and IV have relatively high affinity for  $\text{Ca}^{2+}$  ( $K_{\text{Ca}} = 2 \times 10^7 \text{ M}^{-1}$ ) and also bind  $\text{Mg}^{2+}$  ( $K_{\text{Mg}} = 2 \times 10^3 \text{ M}^{-1}$ ). The two binding sites of the N-domain (sites I and II) are specific to  $\text{Ca}^{2+}$  and are of lower affinity. In the literature, controversial results may be found regarding the binding affinity and the cooperativity of the low affinity sites. Some groups report cooperative coupling between sites I and II, while others propose that the binding process is sequential. The determined binding affinity varies between  $10^4$  and  $10^5$ . A recent detailed NMR study on NTnC has quantitatively determined the binding constants in sites I and II [10]. A  $\text{Ca}^{2+}$  titration which was monitored by 2D- $(^{15}\text{N}, ^1\text{H})$  HMQC experiments has revealed a stepwise binding for NTnC with affinities ( $K_{\text{Ca}}$ ) of  $5.3 \times 10^5$  and  $5.7 \times 10^4 \text{ M}^{-1}$ .

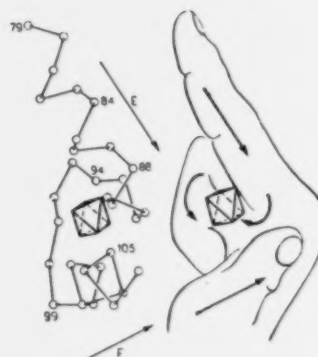


**Figure II-2.** A helix-loop-helix motif can be symbolized by a right hand. The first helix runs from the tip to the base of the forefinger. The flexed middle finger corresponds to the calcium binding loop. The thumb, roughly perpendicular to the forefinger, represents the second helix of the motif. This motif was first observed in the crystal structure of carp parvalbumin where one of the binding sites is formed by helix E and F, hence the term "EF-hand".



*The crystal structure*

The crystal structures of both chicken and turkey skeletal TnC have been solved [11, 12, 13]. A ribbon representation of the turkey skeletal crystal structure is shown in figure I-9, and a stereo view of the backbone is displayed in figure II-3. These structures reveal two globular domains, connected by a single 31-residue long central helix. Each domain contains two helix-loop helix units coupled via a short  $\beta$ -sheet formed by the two loops. In these crystals,  $\text{Ca}^{2+}$  is bound only in the high affinity sites, whereas sites I and II are  $\text{Ca}^{2+}$ -free. The structure of the  $\text{Ca}^{2+}$ -bound C-domain was found to be very similar to other homologous  $\text{Ca}^{2+}$ -binding

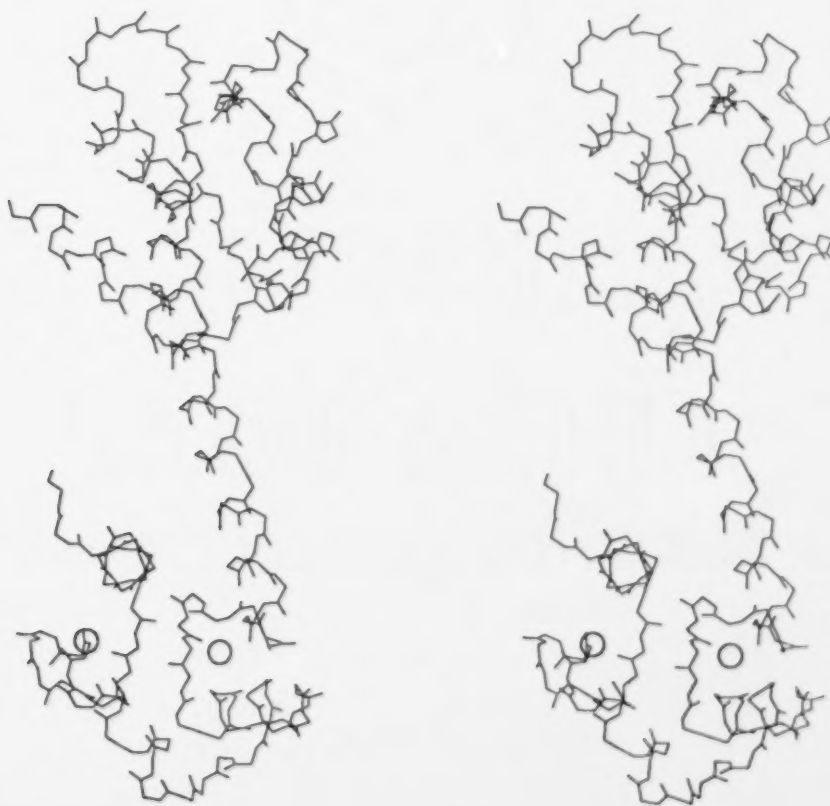


**Figure II-3.** Stereo view of the crystal structure of turkey skeletal troponin-C. Only the backbone N, C $\alpha$ , C, O and the  $\text{Ca}^{2+}$  ions are displayed.

protein structures [14]. The apo N-domain, however, was considerably different mainly in terms of interhelix angles [14]. Table II-2 compares the interhelix angles of the N-domain of TnC with a few  $\text{Ca}^{2+}$ -bound protein structures, and clearly indicates that the A/B and C/D interhelix angles vary considerably from the usual helix-loop-helix organization.

**Table II-2.** Interhelix angle differences between the N-domain of TnC and other  $\text{Ca}^{2+}$ -binding proteins. <sup>(a)</sup>

| HELIX PAIR | others(range) <sup>(b)</sup> | N-domain TnC | Diff <sup>(c)</sup> |
|------------|------------------------------|--------------|---------------------|
| A/B or E/F | 96-109                       | 134          | 32                  |
| B/C or F/G | 111-124                      | 122          | 5                   |
| C/D or G/H | 84-108                       | 146          | 49                  |
| A/D or E/H | 110-123                      | 115          | 2                   |

(a) interhelix angles obtained from ref. 14.

(b) includes TnC (C-domain only), calmodulin (2 pairs) and parvalbumin (1 pair).

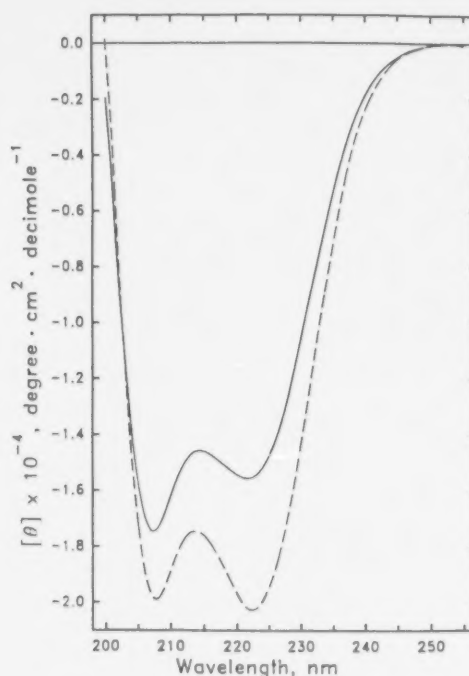
(c) difference between the N-domain of TnC and the average of the four related pairs.

## **Ca<sup>2+</sup>-induced conformational changes**

### *Evidence of conformational changes*

Evidence of the Ca<sup>2+</sup>-induced conformational changes in TnC was first observed more than two decades ago in the same department as the work presented in this thesis has been done. In 1972 Murray and Kay [15], used CD spectroscopy to demonstrate that the binding of Ca<sup>2+</sup> was inducing a conformational change in troponin-C (or more accurately the ancestor of troponin-C, troponin A). Over the years, proton magnetic resonance spectroscopy has been used extensively to characterize the structural changes of the two domains of TnC. These studies have shown that binding of Ca<sup>2+</sup> to the high affinity sites (C-domain) induces changes in the protein fold, and that binding to the low affinity sites (N-domain) leads mainly to changes among hydrophobic side-chains [16, 17, 18, 19, 20, 21]. Laser Raman spectroscopy was also used, and revealed a large increase in  $\alpha$ -helical content due to Ca<sup>2+</sup>-binding to the C-domain sites, and none associated to the N-domain [22]. Other studies, using CD and fluorescence, reported large spectroscopic changes associated with Ca<sup>2+</sup>-binding to the C-domain, and small changes when the low affinity sites were filled [23, 24, 25, 26, 27]. These studies reported a large increase of negative ellipticity when sites III and IV are filled, consistent with the NMR results. The estimates of the magnitude of the Ca<sup>2+</sup>-induced far-UV CD ellipticity changes attributable to the N-domain transition were more subtle, less clearly defined, and somewhat contradictory. A recent characterization of the Ca<sup>2+</sup>-induced spectroscopic changes of the recombinant NTnC clearly indicated a significant increase in the negative far UV CD ellipticity [28] (figure II-4). These data suggest a significant Ca<sup>2+</sup>-induced transition involving secondary structural elements of the N domain.

**Figure II-4.** Far-UV CD spectra of recombinant N-domain of TnC in the absence (—) and presence of  $\text{Ca}^{2+}$  ( $\text{pCa} = 3.8$ ) (---). The binding of  $\text{Ca}^{2+}$  induces a very significant increase (~23%) in the negative values of  $[\theta]_{221\text{nm}}$ .



#### *Model for the $\text{Ca}^{2+}$ -induced structural change*

By comparing the crystal structure of the apo N-domain of TnC with its homologous C-domain, Herzberg et al. proposed a model for the conformational change which occurs in the N-domain of TnC upon  $\text{Ca}^{2+}$  binding [29]. Using molecular modeling and the assumption that the  $\text{Ca}^{2+}$ -filled N-domain would adopt a conformation similar to the  $\text{Ca}^{2+}$ -filled C-domain, they deduced a tentative structure of TnC in the  $4\text{Ca}^{2+}$  state. The major conformational change in their model is the movement of the B/C pair of helices away from the A/D pair, therefore exposing a hydrophobic patch (see figure II-5). This model is the only structural representation of the  $\text{Ca}^{2+}$ -saturated form of the regulatory domain of TnC.

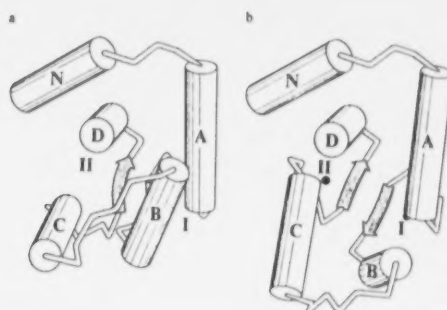


**Figure II-5.** Diagrammatic representation of the proposed  $\text{Ca}^{2+}$ -induced conformational change in the N-terminal domain of TnC [29]. In this model helices N, A, and D retain their relative dispositions. Helices B and C and the linker peptide move by up to 14 Å when  $\text{Ca}^{2+}$  binds. The relative dispositions of helices B and C also remain constant. (a)  $\text{Ca}^{2+}$ -free conformation of the N-terminal domain of TnC. (b) Proposed  $\text{Ca}^{2+}$ -bound conformation of this domain.

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CHAPTER **III****NMR FOR STRUCTURAL  
CHARACTERIZATION**

*The circumstances of the discovery of magnetic properties are veiled in the clouds of antiquity. Passages in the ancient annals of the Chinese Empire refer to a certain "south-pointing carriage" which guides men in fog or storm (~ 2637 B.C.). However, archaeological researches show that these carriages had nothing to do with the magnetic compass but were assemblies of gear wheels. 2000 years later, Thales of Miletus (640-546 B.C.), the Father of Philosophy, is said to have attributed a soul to a magnet in order to explain its attractive property. More recently, the text Meng Chhi Pi Than (Dream Pool Essays) dating about A.D., 1080 written by the Chinese philosopher Shen Kua says, "Magicians rub the point of a needle with the lodestone, after which it is able to point to the south".*

*Although the origin of the discovery of magnetic properties is uncertain, the discovery of the nuclear magnetic resonance phenomenon is well known. It was in 1946 that Bloch at Stanford and Purcell at Harvard, working independently, were able to demonstrate that nuclei can absorb electromagnetic radiation in a strong magnetic field. The two physicists shared the 1952 Nobel prize for their work. A second Nobel prize was awarded in 1991, this time to Richard R. Ernst for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy.*

*As for the safety related to long-term exposure to high magnetic fields, NMR spectroscopists can be reassured. Experiments were made in 1892 by Thomas Alva Edison and his collaborators in which they subjected themselves and one dog to very strong magnetic fields without sensing any effect.*

## General theory

### *Spins in a magnetic field*

Many nuclei behave as if they were spinning charges. These are typically nuclei with odd mass ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{31}\text{P}$ , etc.) and/or odd atomic number ( $^2\text{H}$ ,  $^{14}\text{N}$ , etc.), which possess a quantized spin angular momentum and a magnetic moment. Each nuclei with spin has a nuclear spin quantum number  $I$  associated with it. For a nucleus of spin quantum number  $I$ , there are  $2I+1$  allowed spin states (from  $-I$  to  $+I$ ). Since the nuclei encountered in this thesis ( $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$ ) all have a spin quantum number  $I=1/2$ , only this case will be discussed. Therefore these nuclei have two allowed spin states:  $-1/2$  and  $+1/2$ . In the absence of a magnetic field, the two spin states have the same energy and are equally populated.

When in an applied magnetic field, however, the two spin states are not of equivalent energy. This is due to the fact that a nucleus is a charged particle, and that any moving charge generates a magnetic field of its own. In a magnetic field, all protons will have the  $z$  component of their magnetic moment either aligned to the applied magnetic field, or opposed to it (figure III-1). Hence, upon application of an external magnetic field, the

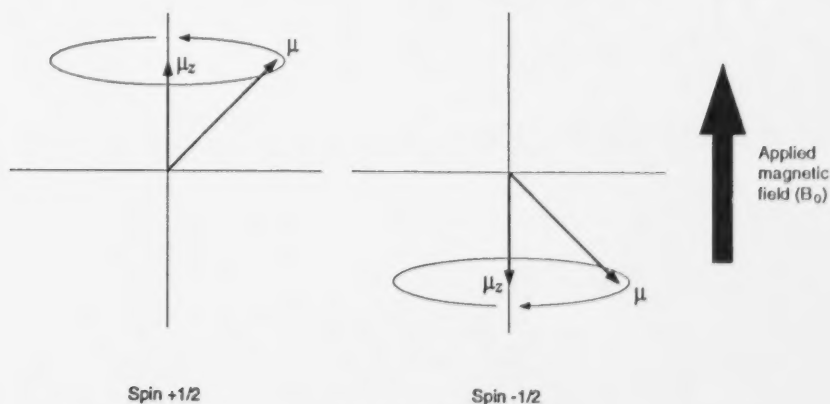


Figure III-1. The two allowed spin states for a nucleus of spin  $1/2$ .

degenerate spin states split into two states of unequal energy (figure III-2).

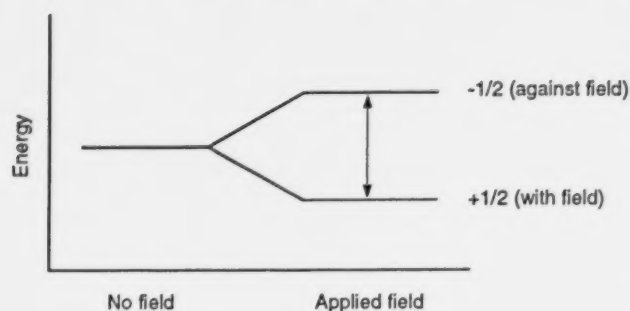


Figure III-2. The energy separation of a spin 1/2 in a magnetic field.

The NMR phenomenon occurs when nuclei absorb energy and change their spin state. The energy absorbed (ie. the energy difference between the two states) is quantized. This  $\Delta E$  is proportional to the applied magnetic field  $B_0$  and is also dependent on the particular nuclei involved. The energy difference between two spin states is given by:

where  $h$  = Planck's constant

$\nu$  = the frequency of resonance

$\gamma$  = gyromagnetic ratio, a  
characteristic of the particular  
nucleus

$$\Delta E = h\nu = \left(\frac{h}{2\pi}\right)\gamma B_0$$

Energy is absorbed by nuclei because of the fact that, in an applied magnetic field, nuclei precess about their own axis of spin with an angular frequency  $\omega$ , which is called the Larmor frequency. The angular frequency,  $\omega$ , is directly proportional to the applied magnetic field. For example, a proton in a 141 000 Gauss field has a frequency of precession of approximately 600 MHz. Energy can be absorbed if radiofrequency waves of this same frequency are applied. This absorption phenomenon is called resonance.

*From a single spin to many spins*

So far I have been talking about a single spin in a static field. However, what we actually observe is the macroscopic magnetization, or the sum of all the microscopic transitions. Therefore I will now discuss and refer to the bulk magnetization,  $M$ . At equilibrium in a static field  $M$  is perfectly aligned with the applied magnetic field, as the  $xy$  components of the individual spins cancel out (figure III-3a).

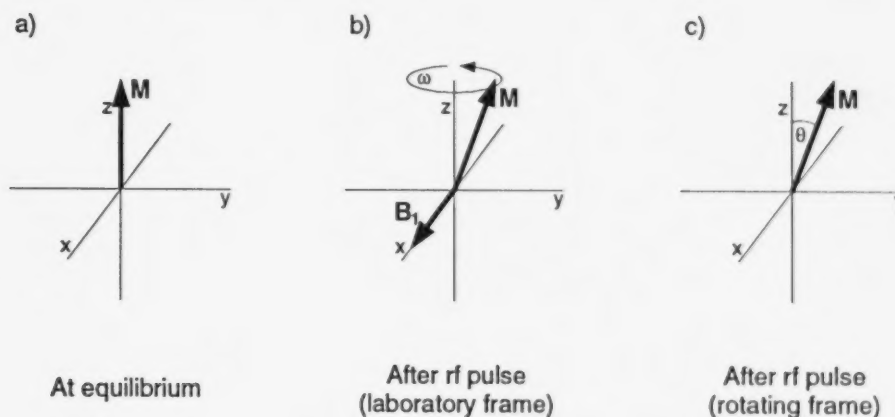


**Figure III-3.** Application of a pulse. a) Bulk magnetisation  $M$  at equilibrium in a magnetic field applied along the  $z$  axis. b) Magnetisation  $M$  after the application of a short pulse along the  $x$  axis, as viewed in the static laboratory frame. c) Same as b), but in a rotating frame precessing at frequency  $\omega$ .

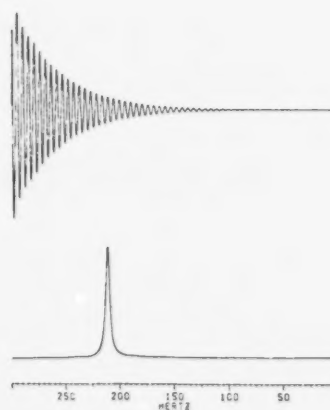
*Observation of NMR phenomenon*

There are three major components in any NMR experiment: pulses, delays and acquisition. Pulses refer to the application of radiofrequency (rf) waves for a certain delay (typically in the 1-100  $\mu\text{s}$  range). An rf pulse usually covers the range of frequencies that are to be observed. If an rf pulse is applied for a short period of time in such a way that it produces a second magnetic field  $B_1$  which is perpendicular to the field  $B_0$ , the bulk

magnetization will be perturbed and will precess at the Larmor frequency (see figure III-3b). If, instead of looking at  $\mathbf{M}$  in the fixed laboratory reference frame, we look at it in a rotating frame which also precesses at the Larmor frequency, we would observe a static  $\mathbf{M}$  which is tilted by  $\theta$  degrees relative to the  $z$  axis. The angle  $\theta$  ("flip angle") is proportional to the length of the pulse  $t_p$  and to the magnitude of  $\mathbf{B}_1$ .

The application of a  $90^\circ$  pulse on the  $x$  axis will bring the magnetization  $\mathbf{M}$  on the  $y$  axis. Having a coil in the  $xy$  plane allows the recording of the oscillating current generated by the precessing magnetization. The system, however, goes back to equilibrium, with the  $xy$  magnetization slowly fading and the  $z$  magnetization growing. The result recorded is shown in figure III-4 and is called a free-induction decay (FID). The FID is a signal oscillating at a frequency corresponding to precession frequency of the nucleus minus a reference frequency ( $\omega - \omega_{ref}$ ). Using a mathematical operation called Fourier transform (FT), the time domain is transformed into a frequency domain in order to obtain an NMR spectrum (figure III-4). Of course in real life, more than one proton would be observed. The FID and one-dimensional  $^1\text{H}$  spectrum of NTnC are shown in figure III-5.

**Figure III-4.** The NMR observed signal is an FID (time domain) which is Fourier Transformed to produce an NMR spectrum (frequency domain).



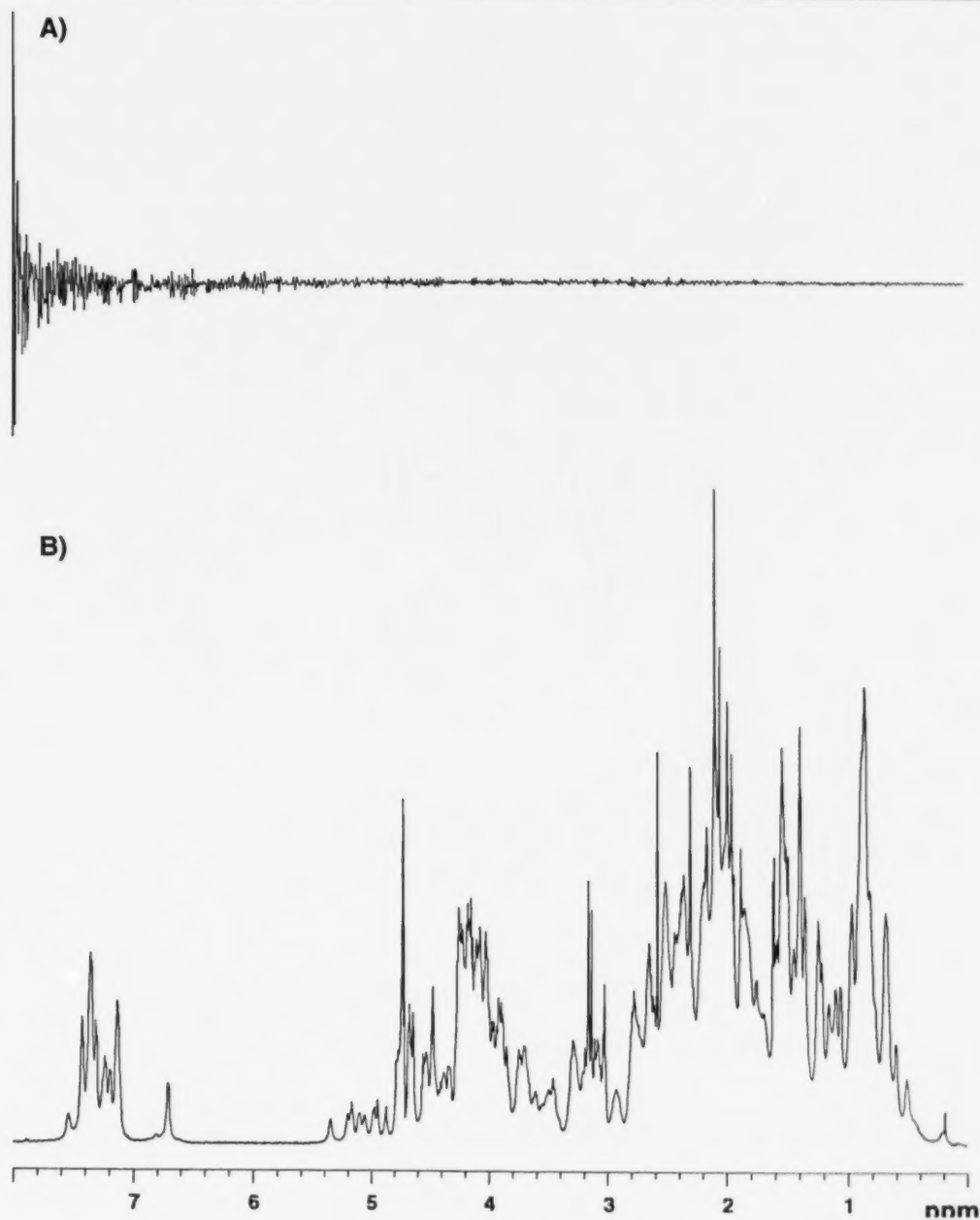


**Figure III-5.** Real life FID (A) and its corresponding spectrum (B) obtained after Fourier Transform. Shown here is a  $^1\text{H}$  spectrum of NTnC complexed with  $\text{Ca}^{2+}$  acquired in  $\text{D}_2\text{O}$ . One-dimensional spectra of proteins are typically very crowded (this spectrum contains over 550 protons).



As will be seen in chapter IV, NMR experiments typically contain many pulses which are often separated by delays. The complete description of the effect of the combination of various pulses and delays will not be given in this thesis, as it would exceed the size and objectives of this thesis, which is the resolution of the solution structure of  $\text{Ca}^{2+}$ -saturated NTnC and the characterization of its  $\text{Ca}^{2+}$ -induced structural changes. I will, however, briefly discuss the two other types (ie. other than chemical shift) of NMR phenomena extensively used in this thesis: through-bond correlation and through-space correlation.

### *Chemical shift*

Of course, NMR would be useless to chemists and biochemists if all protons (or other nuclei) would be precessing at the same frequency. In a molecule, different protons generally have different electronic environments. This is due to circulating electrons which also generate small magnetic fields of their own. A different electronic environment leads to a different effective magnetic field sensed by a given nucleus, and that nucleus will resonate at a different frequency. Therefore, protons in a 141 000 Gauss field will not all resonate at 600 MHz, but actually in the range  $600\,000\,000 \pm 6000$  Hz. This shielding (or deshielding) effect is measured in Hertz and is usually reported relative to a certain reference compound. Since it is dependent on the strength of the applied field, this shift is divided by the frequency at which the spectrometer operates. The result is a field-independent measure, called the chemical shift ( $\delta$ ) with units of ppm (parts per million):

$$\delta (\text{ppm}) = \frac{\text{shift in Hz from a reference}}{\text{spectrometer frequency in MHz}}$$

Proton chemical shifts are typically found within a 15 ppm window.



*Through-bond correlation*

Nuclei are not antisocial and they actually do "talk" to each other. One of the communication modes, or correlations, that can be observed by NMR is a through-bond correlation called J-coupling. This correlation occurs only between nuclei that are connected by a certain number of bonds (typically 1-3 bonds). The magnitude of this coupling is referred to as the coupling constant J (in Hz), and can vary from 0 to a few hundred Hertz. The simplest through-bond correlation experiment used in this work is the 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC, a heteronuclear experiment (correlates two different kinds of nuclei). This experiment also introduces the concept of multi-dimensional NMR spectroscopy.

In the multi-dimensional experiments used in this work, chemical shifts are detected more than once, and the chemical shifts from one dimension are correlated to the chemical shifts of the other(s) dimension(s) using a mode of communication determined by the pulse sequence. In the case of the 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC, the first dimension (F1) reports  $^{15}\text{N}$  chemical shifts, the second dimension (F2) reports  $^1\text{H}$  chemical shifts, and the correlation observed is a one-bond connectivity (or one-bond coupling) between  $^1\text{H}$  and  $^{15}\text{N}$ . The pulse sequence for this experiment is shown in figure III-6. The resulting 2D spectrum is shown in figure III-7. In a protein, this experiment shows a correlation for each of the backbone amide (plus some side-chain correlations from residues that possess NH or  $\text{NH}_2$  groups).

*Through-space correlation*

The most useful NMR correlation to the structural NMR spectroscopist is without any doubt the through-space correlation. This correlation, named the NOE (nuclear Overhauser effect), occurs between nuclei that are close

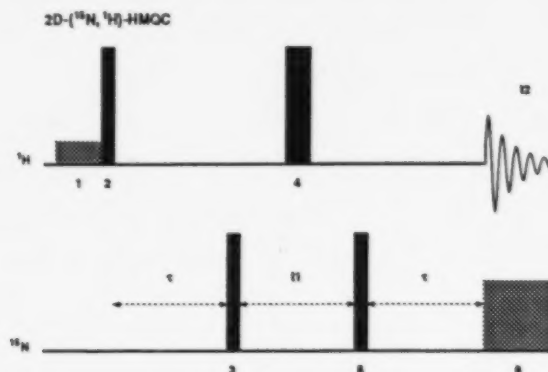


**Figure III-6.** Pulse sequence of the 2D-([<sup>15</sup>N, <sup>1</sup>H])-HMQC experiment. Narrow and large bars indicate 90° and 180° pulses, respectively. Shaded squares represent decoupling periods. The <sup>15</sup>N chemical shifts are recorded during the evolution time t1 and <sup>1</sup>H chemical shifts are detected during the t2 evolution time. More details about this experiment is given in chapter IV.

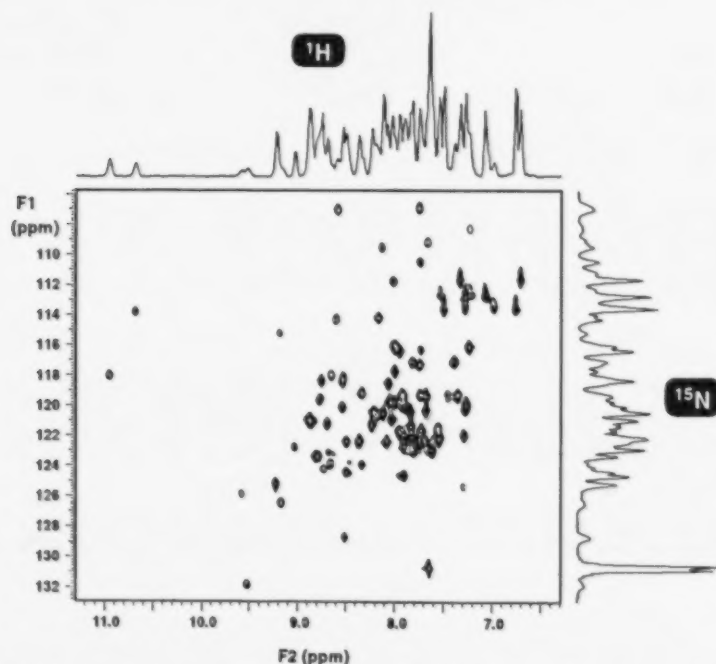


**Figure III-7.** Two-dimensional [<sup>15</sup>N, <sup>1</sup>H])-HMQC acquired using the pulse sequence of figure III-6. The sample is uniformly <sup>15</sup>N-labelled NTnC (Ca<sup>2+</sup>-saturated). On top is a 1D projection corresponding to the 1D-spectrum of the amide protons. On the right is a 1D projection corresponding to the 1D-spectrum of the amide nitrogens. Each correlation peak therefore represents one backbone amide, or side-chain HN/HN<sub>2</sub> group.

in space. In simplest terms the magnitude of the NOE is proportional to  $1/r^6$ , where  $r$  is the distance between two nuclei.

$$\text{NOE} \propto \frac{1}{r^6}$$

Due to this relationship, the NOE fades quickly with distance, and NOE's are not normally observed between protons that are more than 5 Å apart. The pulse sequence for a conventional 2D-NOESY experiment is shown in figure III-8, and the resulting spectrum is displayed in figure III-9. In the homonuclear 2D-NOESY,  $^1\text{H}$  chemical shifts are detected in both dimensions (F1 and F2), and the magnitude of the correlations observed is inversely proportional (to the sixth power) to the distance between the protons at chemical shift F1 and the protons at chemical shift F2.

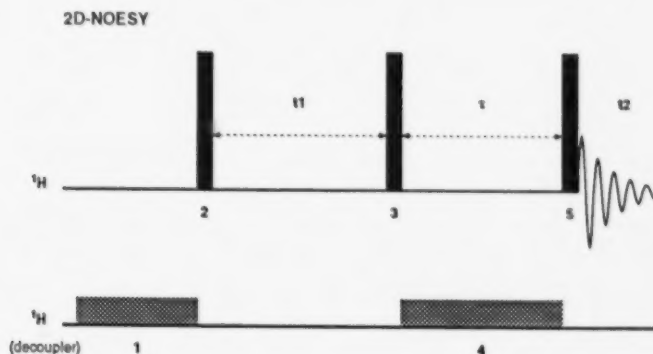


**Figure III-8.** Pulse sequence of the 2D-NOESY experiment. Here proton chemical shifts are recorded both during  $t_1$  and  $t_2$ . The correlation measured in this experiment is the NOE between different protons. The NOE correlation develops during the mixing time ( $\tau$ ).

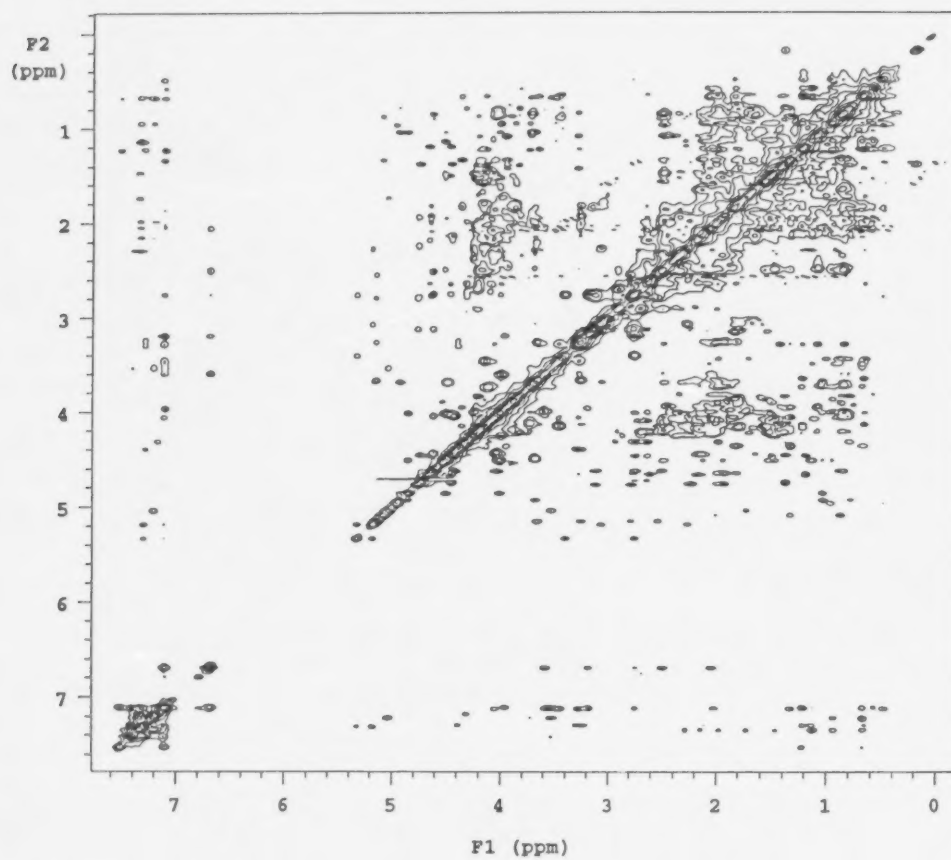


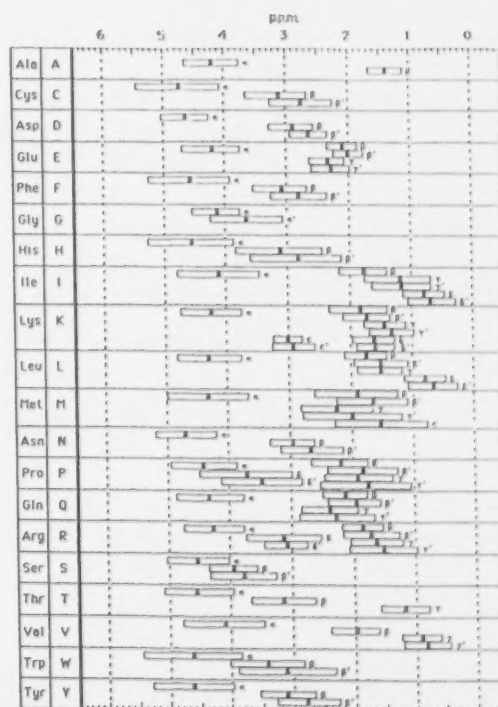
Figure III-9. Two-dimensional NOESY spectrum obtained from the pulse sequence of figure III-8. In this spectrum, both dimensions (F1 and F2) represent  $^1\text{H}$  chemical shifts. The peaks observed correlate to distances between protons in F1 and F2.

## From NMR spectra to solution structure

### *Chemical shift assignment*

The first step of solving an NMR structure is the determination of all the chemical shifts of interest. This step is often the most time consuming (the complete assignment of NTnC involved about 1000 nuclei to be assigned) but also probably the most entertaining (for people who like to solve puzzles). A description of the approach I used will be given in chapters V and VI for the backbone and side-chain assignments, respectively. Through-bond correlation experiments are primarily used for the assignment, but NOE data are usually also necessary for complete assignments. The chemical shift distribution in proteins is reasonable, as can be seen in figure III-10, but chemical shift overlap is inevitable in a large proteins (see figure III-5b).

**Figure III-10.** Distribution of  $^1\text{H}$  chemical shifts in proteins. The horizontal bars correspond to the range of chemical shifts within 1 standard deviation of the mean, and the dark portion is the actual average chemical shift. Statistics like the one illustrated in this figure are extremely useful to help assign protein NMR spectra.



### Secondary structure determination

Once most of the backbone resonances and backbone NOE's are assigned, the secondary structure of a protein can be determined. This can usually be accomplished via two methods, which may be combined to give an accurate determination of the secondary structural features of a protein. The first method involves interpretation of the NOE data, and is based on the fact that different secondary structure stretches have different NOE patterns (or different interproton distances). This approach has been developed by Kurt Wüthrich [7], and is illustrated in figure III-11. The second approach, known as the chemical shift index (CSI), relies solely on the chemical shifts of the backbone nuclei, and has been optimized by Wishart et al. [4, 5, 6]. The basis of this approach is clearly illustrated in figure III-12 and III-13, showing the relationship between chemical shifts and protein secondary structure. The chemical shift statistics compiled by David Wishart [4, 5, 6] has played an important role in both the assignment and the secondary structure determination stage. More details on the secondary structure determination will be given in chapter V.

|                                        | $\beta, \beta_p$           | $\alpha$ -Helix                | $3_0$ -Helix                 | Turn I         | Turn II        | Turn I'        | Turn II'       | Half-Turn      |
|----------------------------------------|----------------------------|--------------------------------|------------------------------|----------------|----------------|----------------|----------------|----------------|
| $d_{\alpha N}(i, i+4)$                 |                            | =====                          |                              |                |                |                |                |                |
| $d_{\alpha\beta}(i, i+3)$              |                            | =====                          | =====                        |                |                |                |                |                |
| $d_{\alpha N}(i, i+3)$                 |                            | =====                          | =====                        | =====          |                | =====          | =====          |                |
| $d_{NN}(i, i+2)$                       |                            | =====                          | =====                        | =====          | =====          | =====          | =====          |                |
| $d_{\alpha N}(i, i+2)$                 |                            |                                | =====                        | =====          | =====          | =====          | =====          | =====          |
| $d_{NN}$                               | =====                      | =====                          | =====                        | =====          | =====          | =====          | =====          | =====          |
| $d_{\alpha N}$<br>$3J_{HN\alpha}$ (Hz) | =====                      | =====                          | =====                        | =====          | =====          | =====          | =====          | =====          |
|                                        | 9 9 9 9 9 9<br>1 2 3 4 5 6 | 4 4 4 4 4 4 4<br>1 2 3 4 5 6 7 | 4 4 4 4 4 4 4<br>1 2 3 4 5 6 | 4 9<br>1 2 3 4 | 4 5<br>1 2 3 4 | 7 5<br>1 2 3 4 | 7 9<br>1 2 3 4 | 4 9<br>1 2 3 4 |

Figure III-11. Survey of expected NOE's in various secondary structures [7]. The thickness of the lines represents the NOESY cross-peak intensities.



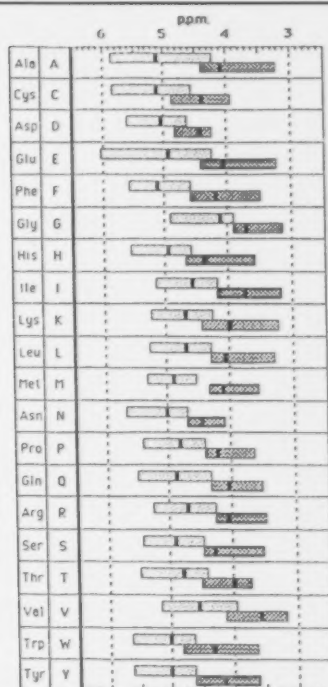


**Figure III-12.** Distribution of  $\alpha$ -proton chemical shifts in helices (dark bars) and  $\beta$ -strands (light bars).  $\alpha$ -proton chemical shifts can therefore be used to estimate secondary structure.

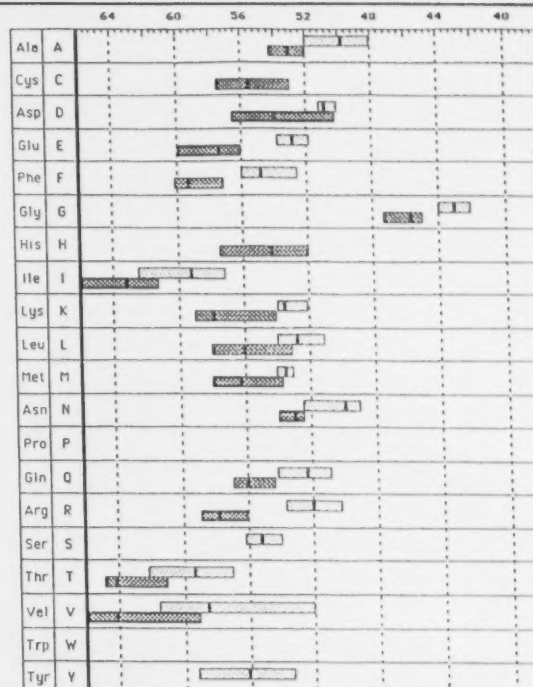


**Figure III-13.** Distribution of  $\alpha$ -carbon chemical shifts in helices (dark bars) and  $\beta$ -strands (light bars). Similar to  $\alpha$ -protons,  $\alpha$ -carbon chemical shifts are useful for characterizing secondary structure.

### *Tertiary solution structure determination*

After all the resonances in the protein have been assigned, as many NOE's as possible must be subsequently assigned. Nowadays, this step is usually done in a semi-automated way with the help of computer software. Details regarding the assignment of over 1000 NOE's will be briefly given in chapter VI. This NOE data is then transformed into distance information and fed to a structure calculation program. Typically 20-60 structures are calculated in order to cover most of the allowed conformational space that agrees with the experimental data. Figure III-14 summarizes the various steps involved in protein NMR structural studies.



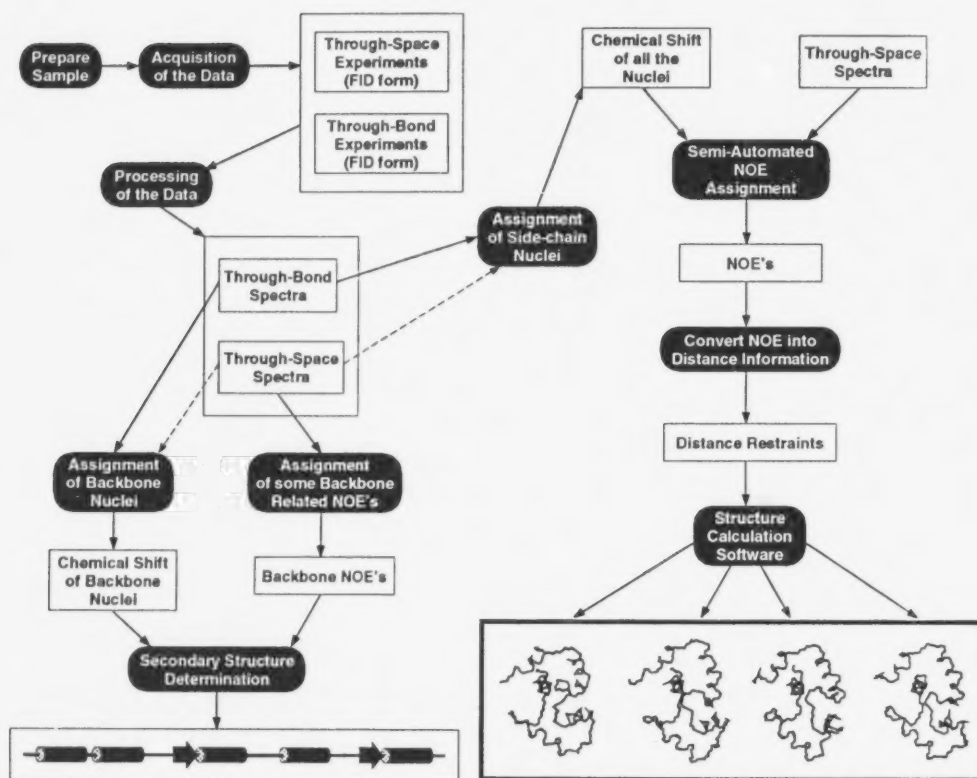


**Figure III-14.** Schematic diagram of the various steps involved in a typical protein structure determination by NMR. Rounded boxes/reverse text represents processes, and squared boxes/black text represent input or output from a process. Dashed arrows represent a minor contribution to the corresponding process.

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- 2 Jeener, J., Meier, B.H., Bachmann, P. & Ernst, R.R. (1979). *J. Chem. Phys.* **71**, 4546-4553.
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CHAPTER **IV****GETTING THE DATA**

*Two-dimensional NMR has been used in recent years as a structural tool for small proteins [1]. Since spectral overlap is proportional to the size of the protein studied, analysis of larger proteins using 2D-NMR becomes tedious and structure determination is limited to a fairly low resolution. With the development of multidimensional heteronuclear NMR (3D- and 4D-NMR) in the last few years [2], and the feasible  $^{15}\text{N}/^{13}\text{C}$  labeling of proteins via cloning and expression, NMR is now a powerful structural method for proteins in the 10-20 kDa range. These 3D- and 4D-NMR techniques allow removal of most of the overlap in NMR spectra, resulting in more complete analyses and high resolution solution structures.*

## First Things First: Getting the Protein

### *NTnC preparation and $^{13}\text{C}$ and $^{15}\text{N}$ labeling*

Although I was not involved in the preparation of the protein I used during my research, that protein did not fall from the sky. I believe it is essential to include the procedure behind the preparation of NTnC simply because... no protein  $\equiv$  no structure. On this note, I reiterate my acknowledgment to the people in Dr. Smillie's lab, especially to Monica X. Li who produced ~400 mg of uniformly labelled  $^{15}\text{N}$ -NTnC and ~200 mg of uniformly double-labelled  $^{15}\text{N}/^{13}\text{C}$ -NTnC.

For the preparation of NTnC the polymerase chain reaction was performed by using two 30 mer oligonucleotides and pTZ18.TnC (91stop) as a template. The construction of pTZ18.TnC (91 stop) was described in Li et al. [3]. The 5'-end oligonucleotide for the polymerase chain reaction was designed to include codons corresponding to amino acid residues 1-6 of TnC (underlined) and flanked on the 5' direction by an initiation codon (bold letters) and nucleotides of the pET3a vector [4] including the NdeI restriction enzyme site:

5'-GAGATATACATATGGCGTCAATGACGGACC-3'.

The 3' end oligonucleotide includes a sequence (underlined) corresponding to amino acid residues 85-90 of the non-coding strand of the TnC gene. This is preceded in the 5' direction by a complimentary sequence for a stop codon (bold letters) and a BamHI restriction site:

5'-AATATGGATCCTAGGCGTCCTCTTTCATCT-3'.

Polymerase chain reaction was performed in a Perkin Elmer thermal cycler (model 480) using Taq polymerase and conditions essentially as described by Ho et al. [5]. The amplified DNA fragment was digested with restriction enzymes NdeI and BamHI and ligated into the corresponding sites of expression vector pET3a plasmid DNA cleaved with the same enzymes [4]. The ligation mixture was transformed into competent *E.Coli*

BL21(DE3) pLys S cells and the entire region of the TnC gene sequenced to ascertain the correctness of the amplification by the Taq polymerase enzyme. The expression of NTnC was carried out in minimal medium utilizing the isopropyl  $\beta$ -D-thiogalactopyranoside induction protocol of Studier et al. [4]. The minimal medium consisted of M9 salts as described by Maniatis et al. [6] with  $(\text{NH}_4)_2\text{SO}_4$  replacing  $\text{NH}_4\text{Cl}$ . Each one liter medium at pH 7.5 contained 6g  $\text{Na}_2\text{HPO}_4$ , 3g  $\text{KH}_2\text{PO}_4$ , 0.5g  $\text{NaCl}$  and 1g  $(\text{NH}_4)_2\text{SO}_4$  to which was added 2 ml of mineral mixture (1M  $\text{MgSO}_4$ , 0.1mM  $\text{FeCl}_3$  and 12.5mM  $\text{ZnSO}_4$ ), 1 ml of 100 mM  $\text{CaCl}_2$  and 1 ml of vitamin mixture (0.1g/100ml each of D-biotin, choline chloride, folic acid, niacinamide, D-pantothenic acid and pyridoxal chloride, 0.5g/100ml thiamine chloride, 0.01g/100ml riboflavin, all in  $\text{H}_2\text{O}$ ). Glucose (3g) dissolved in 20ml  $\text{H}_2\text{O}$  was added with supplements of ampicillin and chloramphenicol to final concentrations of 100 $\mu\text{g}/\text{ml}$  and 25 $\mu\text{g}/\text{ml}$  respectively. The above additives were sterilized separately by filtration. For expression of uniformly enriched  $^{15}\text{[N]}$  and/or  $^{13}\text{[C]}$  NTnC, the  $(\text{NH}_4)_2\text{SO}_4$  and/or glucose of the medium were replaced with  $^{15}\text{[N]}$   $(\text{NH}_4)_2\text{SO}_4$  (99.9 atom %) and/or  $^{13}\text{[C]}$  glucose (99.0 atom %) respectively, both purchased from Isotec Inc. Purification of NTnC followed the previously published procedure for fusion TnC [7]. During expression in *E. Coli*, the N-terminal Met, corresponding to the initiation codon, is cleaved off leaving Ala-1 as the N-terminal residue. Overall recovery of NTnC was ~100mg/liter of growth culture.

#### *NMR sample preparation*

Upon reception of the protein, I first removed the  $\text{Ca}^{2+}$  from the sample. To make the calcium-free sample (apo-NTnC), decalcification was accomplished by using G-25 gel filtration: 10mg of the sample dissolved in 1ml of 0.5M EDTA (pH=8.0) was applied to a 160ml (1.5x90cm) G-25 gel filtration column and eluted with 25mM  $\text{NH}_4\text{HCO}_3$ . The profile of the separation is shown in figure IV-1. Fractions containing protein were

pooled, lyophilized, dissolved in  $\text{Ca}^{2+}$ -free water and lyophilized again to volatilize all of the  $\text{NH}_4\text{HCO}_3$ . 10mg of apo-NTnC sample was dissolved in 0.5ml of 100mM KCl in either  $\text{H}_2\text{O}$  ( $\text{H}_2\text{O}:\text{D}_2\text{O}=9:1$ ) or  $\text{D}_2\text{O}$  (99.996%), to give a final concentration of 2mM. 4.2 mM of  $\text{CaCl}_2$  was added to obtain the  $2\text{Ca}^{2+}$  form of NTnC. Upon addition of  $\text{CaCl}_2$  the pH usually drops between pH 5 and 6, and NaOH (or NaOD) was used to adjust the pH up to 6.7. The same procedure was used for the unlabelled, the  $^{15}\text{N}$  and the  $^{15}\text{N}/^{13}\text{C}$  samples.

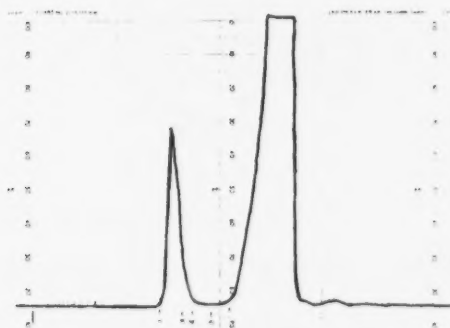


**Figure IV-1.** Profile of the separation on G25 column. The first peak (left) is NTnC and the second peak is EDTA.

## Data Acquisition

### *Overview*

All experiments were carried out on a Varian Unity-600 NMR spectrometer operating at a temperature of 30°C. The following experiments were used for the study of NTnC·2Ca: 2D ( $^{15}\text{N}$ - $^1\text{H}$ ) HMQC [8], 3D-HNCA [9], 3D-HNCO [9], 3D-HNCOCA [9], 3D-HCACO [10], 3D- $^{15}\text{N}$ -edited NOESY (150 ms) [11], and 3D- $^{15}\text{N}$ -edited TOCSY (70 ms) [12]. The  $^3J_{\text{HN-H}\alpha}$  coupling constants were obtained from HMQC-J experiments [18] with the following numbers of complex points and spectral widths: ( $^1\text{H}$ ) 1024, 8000 Hz, ( $^{15}\text{N}$ ) 384, 1800 Hz. For the spectra recorded in  $\text{H}_2\text{O}$ , water suppression was achieved by presaturation (1.0-1.2 sec.).

### *Acquisition and processing*

In all experiments, the  $^1\text{H}$  acquisition dimension was centered at the water frequency (4.67 ppm) with a spectral width of 13.33 ppm (5.00 ppm in the HCACO case). The indirectly detected  $^{15}\text{N}$  carrier position and spectral width were either 119.22 ppm and 28.01 ppm (HNCA, HNCOCA and HNCO), or 117.44 ppm and 23.03 ppm ( $^{15}\text{N}$ -edited NOESY and  $^{15}\text{N}$ -edited TOCSY). The corresponding parameters for the  $^{13}\text{C}\alpha$  were either 55.83 ppm and 30.25 ppm (HNCA, HCACO), or 56.11 and 24.18 (HNCOCA). Finally, the  $^{13}\text{CO}$  carrier position and spectral width were either 177.11 ppm and 11.93 ppm (HNCO), or 176.34 ppm and 18.05 ppm (HCACO).

For the HCACO experiment, the number of complex points acquired was 512 in F3 ( $^1\text{H}$ ); for all other 3D experiments, 1024 complex points were acquired in F3 ( $^1\text{H}$ ). For the HNCA and HNCOCA experiments, there were 48 and 32 complex points acquired in F2 ( $^{13}\text{C}\alpha$ ) and F1 ( $^{15}\text{N}$ ), respectively; the time-domain was increased by 32 complex points in both F2 and F1 dimensions by means of linear prediction and, after zero-filling, the final spectra consisted of  $1024 * 256 * 64$  points. The number of complex points acquired for the HNCO was 64 in F2 ( $^{13}\text{CO}$ ) and 32 in F1 ( $^{15}\text{N}$ ); 32 additional complex points were predicted in the F1 dimension to finally generate, after



zero-filling, a  $1024 * 128 * 64$  point spectrum. For the HCACO, we acquired 35 complex points in F2 ( $^{13}\text{CO}$ ) and 28 complex points in F1 ( $^{13}\text{C}\alpha$ ); both F2 and F1 time-domain were subsequently extended using linear prediction to give a  $512 * 128 * 64$  point spectrum. In the case of the  $^{15}\text{N}$ -edited NOESY and  $^{15}\text{N}$ -edited TOCSY, 32 and 128 complex points were acquired in F2 ( $^{15}\text{N}$ ) and F1( $^1\text{H}$ ), respectively; 32 complex points were added to the F2 dimension by linear prediction to obtain a final  $1024 * 64 * 256$  points spectrum. The HMQC-J's were processed to a final size of  $2048 * 2048$  points with a  $90^\circ$ -shifted sinebell in F2 ( $^1\text{H}$ ) and the following filtering VNMR parameters in F1 ( $^{15}\text{N}$ ): GF=0.06 (NTnC-apo), GF=0.05 (NTnC-2Ca), LB=-8, -10, -12, -14, -16, -18, -20, -22, -24, -26, -28. The splittings at various LB's were fitted with the HMQCJFIT software [19] to obtain the  $^3J_{\text{HNH}\alpha}$  coupling constants.

For all experiments except the HCACO, a post-acquisition solvent suppression by convolution of the time-domain data was applied prior to Fourier transform [13]. In most of the cases, a  $60^\circ$ -shifted squared sinebell was applied in F1 and F2, and a  $90^\circ$ -shifted sinebell function in F3. After Fourier transform of the F3 dimension, parts of the spectra without resonances were discarded, when possible, prior to the processing of F2 and F1, thus reducing the size of the final spectra by 50% or 75%.

Processing of the 2D and 3D data sets was accomplished using either the VNMR software (VNMR 4.1A, Varian, Palo Alto, CA) or NMRPIPE (Delaglio et al., NIDDK, NIH, MD, unpublished). When used within the VNMR software, extension of the time domain was achieved using the linear prediction algorithm *lpfft*. Automatic peak-picking of the transformed 3D spectra was achieved using the CAPP program [14]. Since CAPP is run at the noise level and every spectrum contains imperfections, a number of false peaks are usually picked. Most of those peaks were removed automatically by using the in-house program PPFILT (Willard & Gagné, PENCE, University of Alberta, unpublished). PPFILT filters the peak list of a 3D spectrum through a high signal-to-noise 2D spectrum (or another 3D spectrum). For example, the 3D- $^{15}\text{N}$ -edited NOESY has been filtered through a 2D- $^{15}\text{N}$ -HMQC. In the last step of the peak-picking, we used the

interactive graphic-based program PIPP [14] to visualize the output of CAPP/PPFILT and edit the peak-pick table. Both processing and peak-picking were accomplished on a Sun Sparc2 workstation. The chemical shift reference used for  $^1\text{H}$  and  $^{13}\text{C}$  is 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS).  $^{15}\text{N}$  chemical shifts are reported relative to external acidic  $\text{NH}_4\text{Cl}$  (24.93 ppm).

#### *Pulse sequence programming*

The development of a multitude of three- and four-dimensional experiments occurred in the last few years, as my research project was going along. The three-dimensional pulse sequences used in this study were available only from the literature at the time, and I had to implement them first, along with Carolyn M. Slupsky. Pulse sequences were written in C, the programming language used by the VNMR software to control the spectrometer. The pulse sequence code I wrote will not be listed in this chapter, but can be found in appendix A. The rest of this chapter will present all the pulse sequences implemented and used. Each pulse sequence will be described by:

- 1) Main reference(s)
- 2) Correlation(s) observed by the experiment
- 3) Coupling constant involved in the experiment
- 4) Comments
- 5) Pulse sequence

Note: For all pulse sequences, narrow and large pulse indicate  $90^\circ$  and  $180^\circ$  pulses, respectively. Oval pulses correspond to shifted-laminar pulse [17] Gray-filled rectangular indicate decoupling.

- 6) Phase cycling
  - † indicates pulse shifted  $90^\circ$  for hypercomplex F1
  - ‡ indicates pulse shifted  $90^\circ$  for hypercomplex F2
- 7) Delays
- 8) Spectra

## 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC

**References:** Summers, M.F., Marzilli, L.G. & Bax, A.  
*J. Amer. Chem. Soc.* **108**, 4285-4294 (1986) [15]

Bax, A., Griffey, R.H. & Hawkins, B.L.  
*J. Magn. Reson.* **55**, 301-315 (1983) [16]

**Correlations:** Correlates  $^1\text{HN}$  to covalently bonded  $^{15}\text{N}$ . One peak is found for each observable backbone amide (all amino acids except Pro). Although the backbone correlations are the most used, other peaks appear in the spectra. Gln and Asn side-chains display two additional correlations (one for each proton of the  $\text{NH}_2$  group) which appear like a doublet. Arg side-chains can have up to five additional correlations (one for the  $^1\text{HNE}$  and one for each of the 4  $^1\text{HN}\zeta$ ); these correlations are not always observable due to exchange with the water. Lys, His and Trp can also display side-chain correlations.

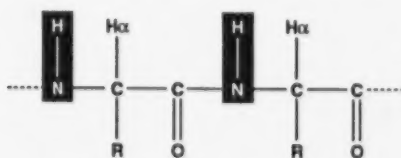


**Figure IV-2:** Correlations observed in 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC

**Coupling constant:**  $^1J_{\text{NH}}$  ~ 95 Hz

**Comments:** This experiment is not used for the assignment purpose

itself. The role of the 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC is rather to provide a first idea of the spectral dispersion and to aid in choosing the  $^{15}\text{N}$  spectral window for the 3D experiments. Folding of  $^{15}\text{N}$  resonances, which is often desired in some 3D-experiments, can be predicted using this experiment. Therefore a 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC with a large spectral window ( $\sim 80$  ppm centered at  $\sim 105$  ppm) should always be one of the first steps in a study involving heteronuclear multidimensional experiments. This experiment can also be used to get accurate  $^1\text{H}$  and  $^{15}\text{N}$  chemical shifts, since the resolution is usually better in this experiment versus 3D-experiments.

*Pulse sequence:*

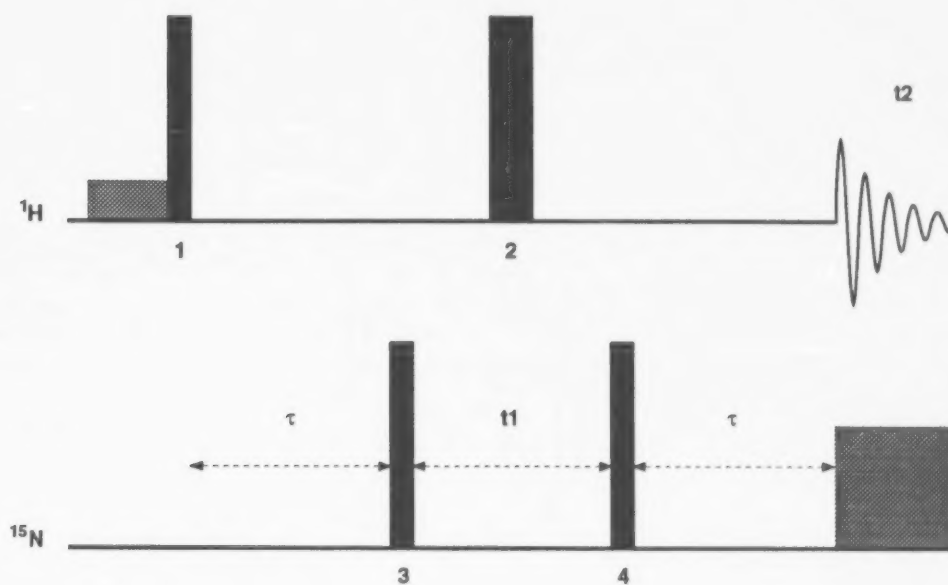


Figure IV-3: Pulse scheme of the 2D- $[^{15}\text{N}, ^1\text{H}]$ -HMQC

*Phase cycling:*

|                |   |    |   |    |
|----------------|---|----|---|----|
| 1              | x | x  | y | y  |
| 2              | x | x  | y | y  |
| 3 <sup>†</sup> | x | -x | x | -x |
| 4              | x | x  | y | y  |
| Acq            | x | -x | y | -y |

*Delay:*

$$\tau \approx 1 / (2 \times {}^1J_{\text{HN}})$$

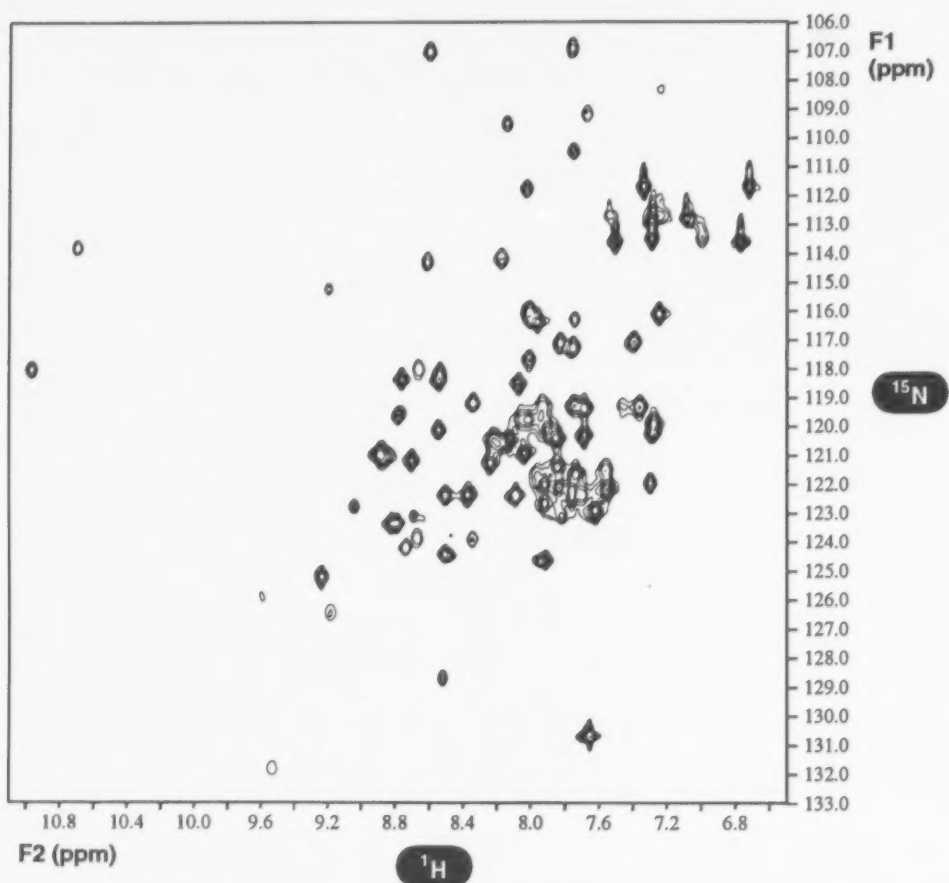


Figure IV-4. 2D-([<sup>15</sup>N, <sup>1</sup>H])-HMQC spectrum of NTnC·2Ca

## 3D-HNCA

**Reference:** Grzesiek, S. & Bax, A.  
*J. Magn. Res.* 96, 432-440 (1992) [9]

**Correlations:** Correlates backbone amides with both the intra-residue  $^{13}\text{C}\alpha$  and the inter-residue (i, i-1)  $^{13}\text{C}\alpha$ .  $^1\text{H}$ ,  $^{15}\text{N}$  and  $^{13}\text{C}\alpha$  resonances are observed. For each  $^1\text{H}/^{15}\text{N}$  pair, there is a strong  $^{13}\text{C}\alpha$  peak for the intra-residue correlation and a weaker  $^{13}\text{C}\alpha$  peak for the inter-residue correlation.

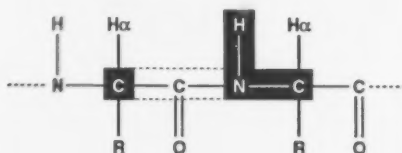


**Figure IV-5:** Correlations observed in 3D-HNCA

**Coupling constant:**

|                         |           |
|-------------------------|-----------|
| $^1J_{\text{NH}}$       | ~ 95 Hz   |
| $^1J_{\text{NC}\alpha}$ | ~ 9-13 Hz |
| $^2J_{\text{NC}\alpha}$ | ~ 5-10 Hz |

**Comments:** This experiment is used for the sequential backbone assignment through the  $^{13}\text{C}\alpha$ .



Pulse sequence:

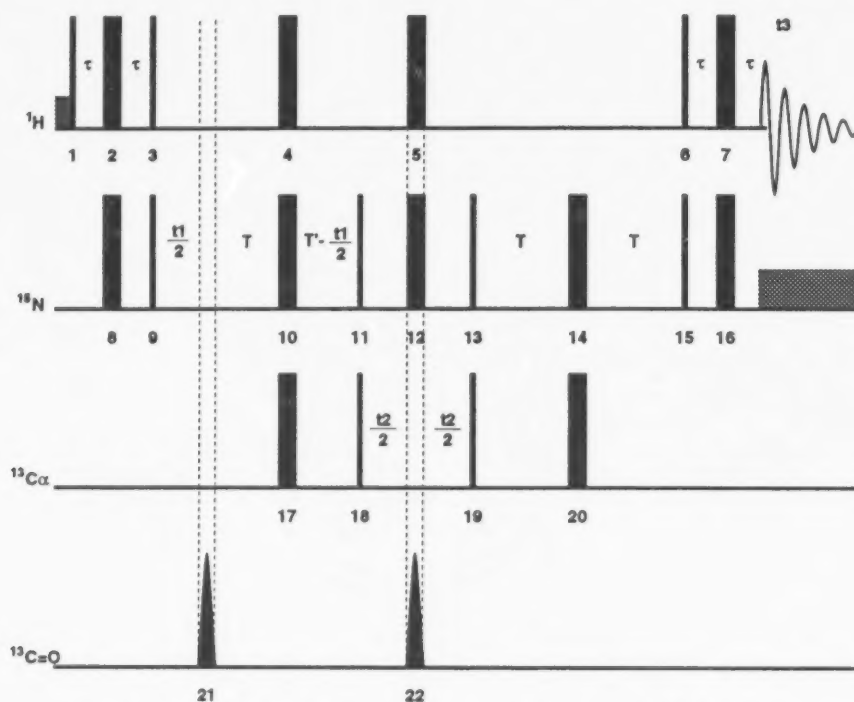


Figure IV-6: Pulse scheme of the 3D-HNCA

Phase cycling:

|    |                  |                  |     |                |     |
|----|------------------|------------------|-----|----------------|-----|
| 1  | x                | 2                | x   | 3              | y-y |
| 4  | x                | 5                | x   | 6              | x   |
| 7  | x                | 8                | x-x | 9 <sup>†</sup> | x   |
| 10 | x x x x y y y y  | -x-x-x-x-y-y-y-y |     |                |     |
| 11 | y y y y y y y y  | y y y y y y y y  |     |                |     |
|    | -y-y-y-y-y-y-y-y | -y-y-y-y-y-y-y-y |     |                |     |
| 12 | x                | 13               | x   | 14             | x   |
| 15 | y                | 16               | x   |                |     |
| 17 | x x x x x x x x  | x x x x x x x x  |     |                |     |
|    | -x-x-x-x-x-x-x-x | -x-x-x-x-x-x-x-x |     |                |     |

---

|                 |                                    |      |      |
|-----------------|------------------------------------|------|------|
| 18 <sup>†</sup> | x x -x -x                          | 19 x | 20 x |
| 21              | x                                  | 22 x |      |
| Acq             | x -x -x x -x x x -x x -x -x x -x   |      |      |
|                 | -x x x -x x -x -x x -x x x -x -x x |      |      |

*Delays:*

$$\tau \approx 1/(4 \times {}^1J_{\text{HN}})$$

$$T = 13.5 \text{ ms}$$

$$T' = T + \text{pulse width (21)}$$

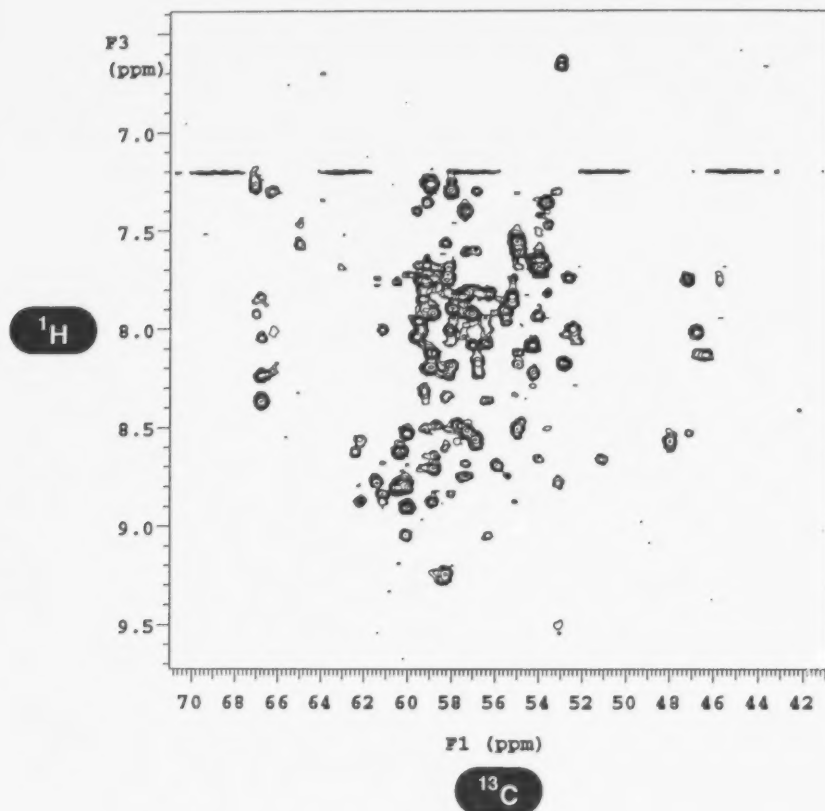


Figure IV-7. Projection of the 64 planes of the HNCA spectrum of NTnC·2Ca.

### 3D-HNCOCA

**Reference:** Grzesiek, S. & Bax, A.  
*J. Magn. Res.* **96**, 432-440 (1992) [9]

**Correlations:** Correlates backbone amides with only the inter-residue (i, i-1)  $^{13}\text{C}\alpha$ .  $^1\text{H}$ ,  $^{15}\text{N}$  and  $^{13}\text{C}\alpha$  are observed. For each  $^1\text{H}/^{15}\text{N}$  pair, there is only one strong inter-residue  $^{13}\text{C}\alpha$  correlation.

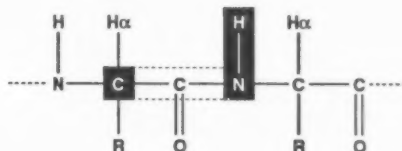


**Figure IV-8:** Correlations observed in 3D-HNCOCA

**Coupling constant:**

|                                 |         |
|---------------------------------|---------|
| $^1J_{\text{NH}}$               | ~ 95 Hz |
| $^1J_{\text{NCO}}$              | ~ 15 Hz |
| $^1J_{\text{C}\alpha\text{CO}}$ | ~ 55 Hz |

**Comments:** This experiment is used for the sequential backbone assignment through the  $^{13}\text{C}\alpha$ .

Pulse sequence:

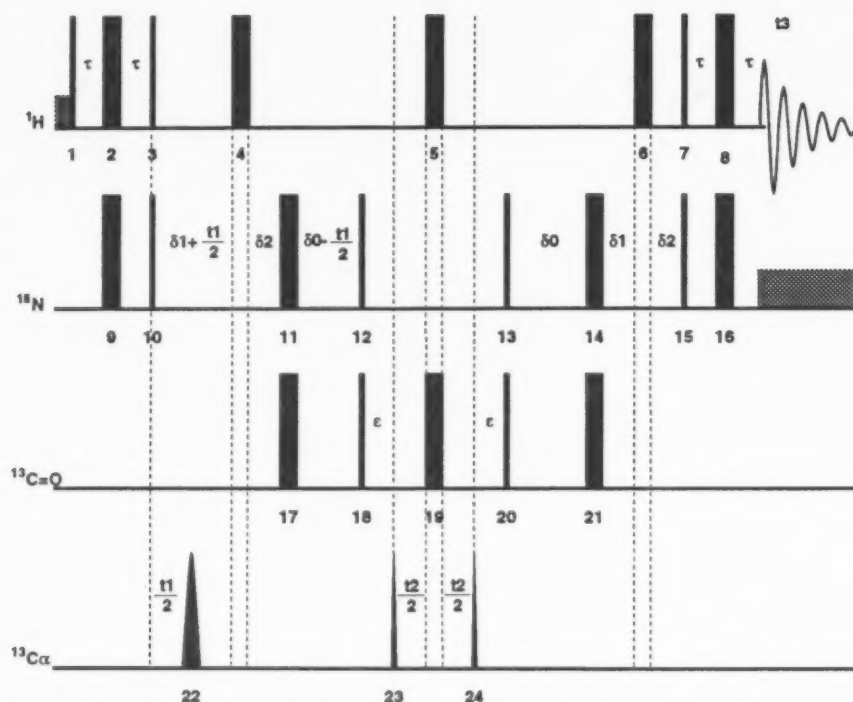


Figure IV-9: Pulse scheme of the 3D-HNCOCA

Phase cycling:

|                 |                 |         |             |                 |           |
|-----------------|-----------------|---------|-------------|-----------------|-----------|
| 1               | x               | 2       | x           | 3               | y-y       |
| 4               | x               | 5       | x           | 6               | x         |
| 7               | x               | 8       | x           | 9               | x-x       |
| 10 <sup>†</sup> | x               |         |             |                 |           |
| 11              | x x x x         | y y y y | -x -x -x -x | -y -y -y -y     |           |
| 12              | x -x            | 13      | x           | 14              | x         |
| 15              | x               | 16      | x           | 17              | x         |
| 18              | x               | 19      | x           | 20              | x         |
| 21              | x               | 22      | x           | 23 <sup>†</sup> | x x -x -x |
| 24              | x               |         |             |                 |           |
| Acq             | x x -x -x -x -x | x x     |             |                 |           |

*Delays:*

$$\tau \approx 1/(4 \times J_{\text{HN}})$$

$$\delta_1 = 7.25 \text{ ms}$$

$$\delta_2 = 2.75 \text{ ms}$$

$$\delta_0 = \delta_1 + \delta_2 + \text{pulse width (22)}$$

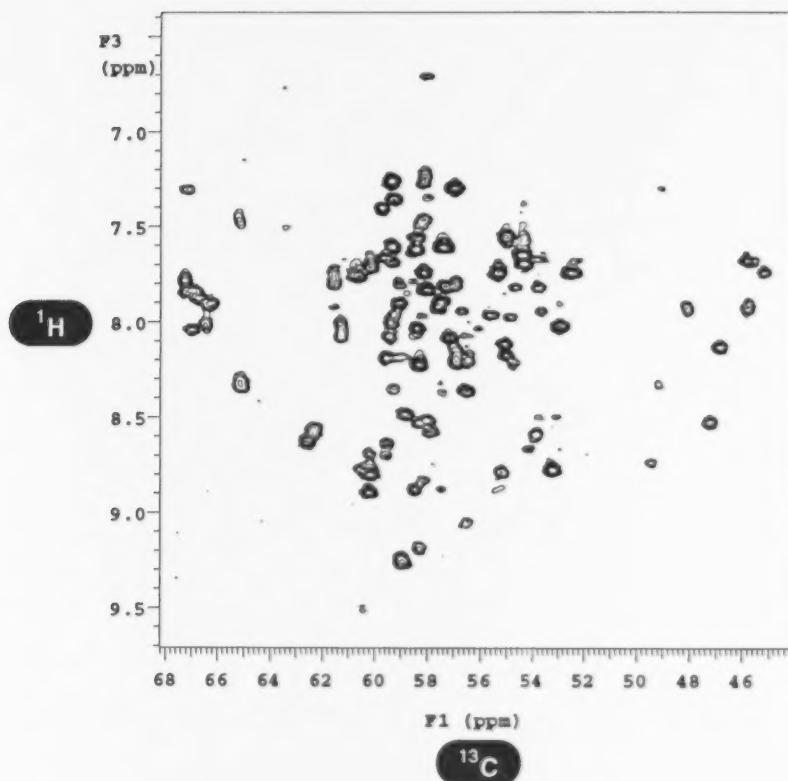


Figure IV-10. Projection of the 64 planes of the HNCOCA spectrum of NTnC·2Ca.



### 3D-HNCO

**Reference:** Grzesiek, S. & Bax, A.  
*J. Magn. Res.* **96**, 432-440 (1992) [9]

**Correlations:** Correlates backbone amides with only the inter-residue (i, i-1)  $^{13}\text{C}=\text{O}$ .  $^1\text{H}$ ,  $^{15}\text{N}$  and  $^{13}\text{C}=\text{O}$  are observed. For each  $^1\text{H}/^{15}\text{N}$  pair, there is only one strong inter-residue  $^{13}\text{C}=\text{O}$  correlation.

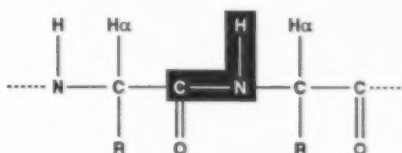


**Figure IV-11:** Correlations observed in 3D-HNCO

**Coupling constant:**

|                    |                      |
|--------------------|----------------------|
| $^1J_{\text{NH}}$  | $\sim 95 \text{ Hz}$ |
| $^2J_{\text{NCO}}$ | $\sim 15 \text{ Hz}$ |

**Comments:** This experiment is used for the sequential backbone assignment through the  $^{13}\text{C}=\text{O}$ .

Pulse sequence:

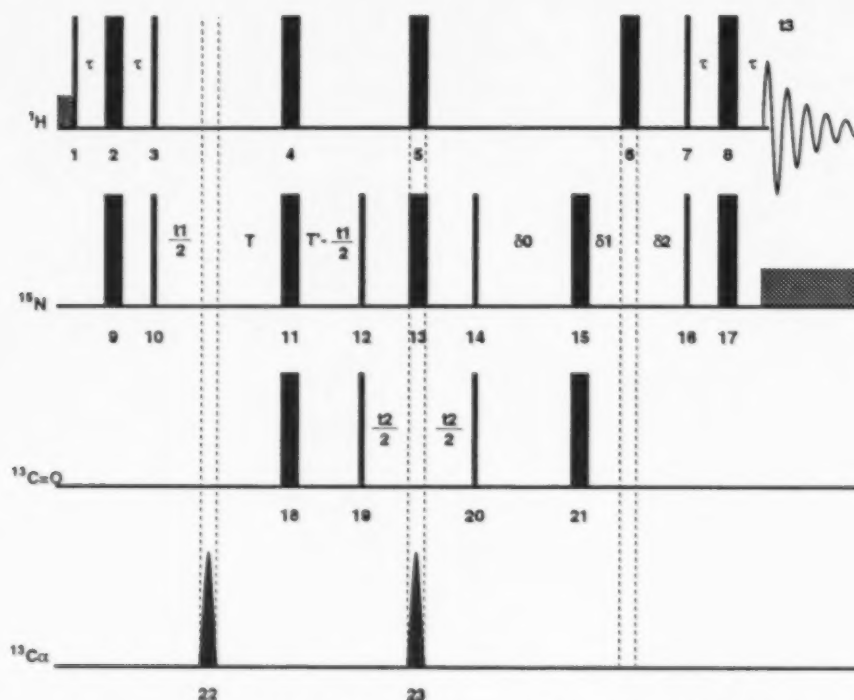


Figure IV-12: Pulse scheme of the 3D-HNCO

Phase cycling:

|                 |                 |                 |             |             |      |
|-----------------|-----------------|-----------------|-------------|-------------|------|
| 1               | x               | 2               | x           | 3           | y -y |
| 4               | x               | 5               | x           | 6           | x    |
| 7               | x               | 8               | x           | 9           | x -x |
| 10 <sup>†</sup> | x               |                 |             |             |      |
| 11              | x x x x         | y y y y         | -x -x -x -x | -y -y -y -y |      |
| 12              | x -x            | 13              | x           | 14          | x    |
| 15              | x               | 16              | x           | 17          | x    |
| 18              | x               | 19 <sup>†</sup> | x x -x -x   | 20          | x    |
| 21              | x               | 22              | x           | 23          | x    |
| Acq             | x x -x -x -x -x | x x             |             |             |      |

*Delays:*

$$\tau = 1/(4 \times {}^1J_{\text{HN}})$$

$$\delta_1 = 7.25\text{ms}$$

$$\delta_2 = 2.75\text{ms}$$

$$\delta_0 = \delta_1 + \delta_2 + \text{pulse width (22)}$$

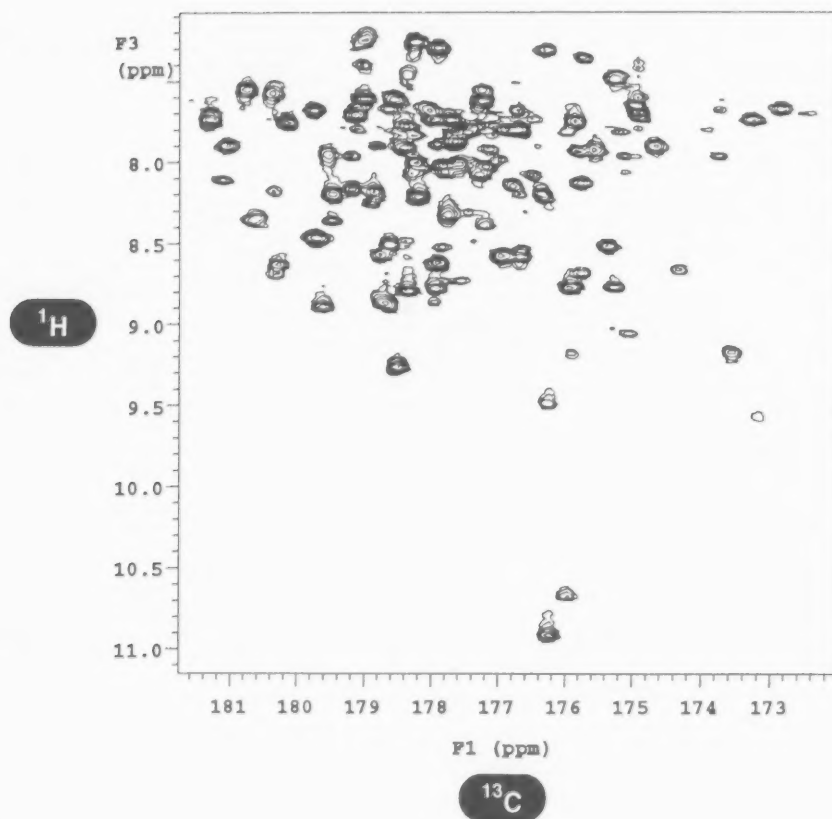


Figure IV-13. Projection of the 64 planes of the HNCO spectrum of NTnC·2Ca.

### 3D-HCACO

*Reference:* Powers, R., Gronenborn, A.M., Clore, G.M. & Bax, A.  
*J. Magn. Reson.* **94**, 209-213 (1991) [10]

*Correlations:* Correlates backbone  $\text{CH}\alpha$  with the intra-residue  $^{13}\text{C}=\text{O}$ .  $^1\text{H}\alpha$ ,  $^{13}\text{C}\alpha$  and  $^{13}\text{C}=\text{O}$  are observed. For each  $^1\text{H}\alpha/^{13}\text{C}\alpha$  pair, there is only one strong intra-residue  $^{13}\text{C}=\text{O}$  correlation.

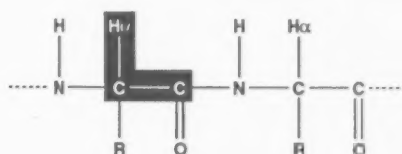


Figure IV-14: Correlations observed in 3D-HCACO

*Coupling constant:*

|                                 |                       |
|---------------------------------|-----------------------|
| $^1J_{\text{CH}}$               | $\sim 140 \text{ Hz}$ |
| $^1J_{\text{C}\alpha\text{CO}}$ | $\sim 55 \text{ Hz}$  |

*Comments:* This experiment is used for the sequential backbone assignment through the  $^{13}\text{C}=\text{O}$ .

Pulse sequence:

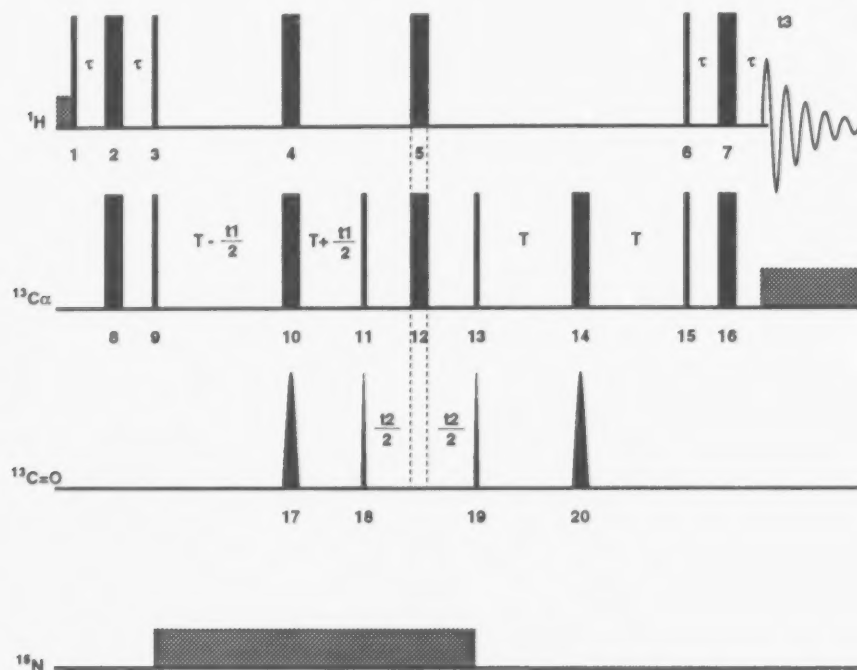


Figure IV-15: Pulse scheme of the 3D-HCACO

Phase cycling:

|                |                                      |    |                     |                 |      |
|----------------|--------------------------------------|----|---------------------|-----------------|------|
| 1              | x                                    | 2  | x                   | 3               | y    |
| 4              | x                                    | 5  | x                   | 6               | x    |
| 7              | x                                    | 8  | x x x x -x -x -x -x |                 |      |
| 9 <sup>†</sup> | x                                    |    |                     |                 |      |
| 10             | x x x x x x x x -x -x -x -x -x -x -x |    |                     |                 |      |
| 11             | y y y y -y -y -y -y                  |    |                     |                 |      |
| 12             | x x x x x x x x -x -x -x -x -x -x -x |    |                     |                 |      |
| 13             | y                                    | 14 | x                   | 15              | x    |
| 16             | x                                    | 17 | x                   | 18 <sup>†</sup> | x -x |
| 19             | x x -x -x                            | 20 | x                   |                 |      |
| Acq            | x -x -x x -x x x -x                  |    |                     |                 |      |

*Delays:*

$$\tau = 1 / (4 \times {}^1J_{\text{HC}})$$

$$T = 3.5 \text{ ms}$$



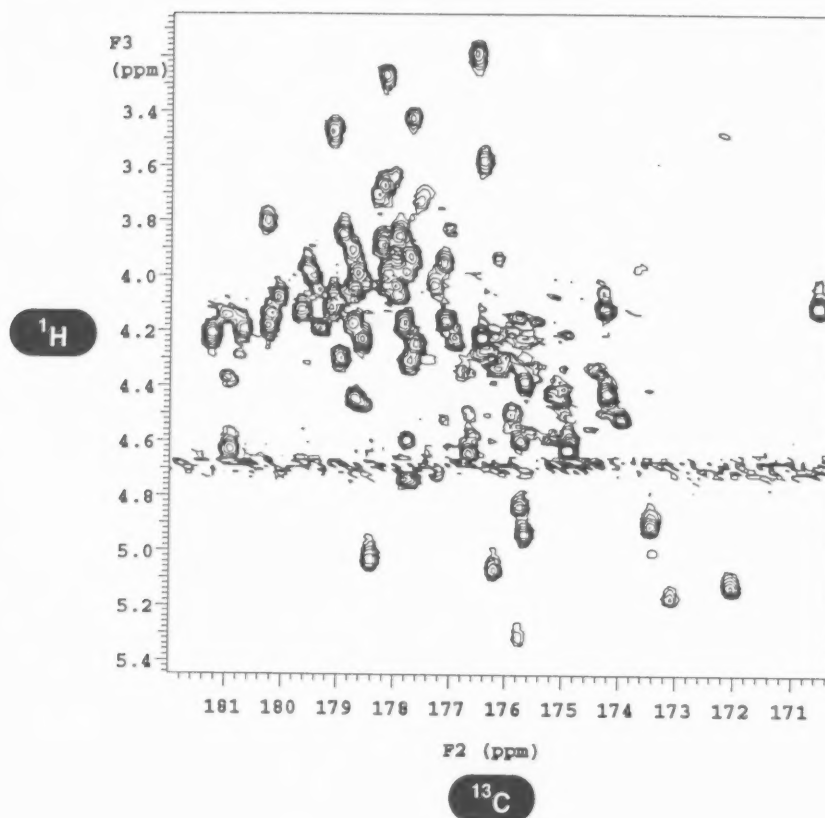


Figure IV-16. Projection of the 64 planes of the HCACO spectrum of NTnC·2Ca.

### 3D- $^{15}\text{N}$ -edited-NOESYHMQC

**Reference:** Kay, L.E, Marion, D. & Bax, A.  
*J. Magn. Res.* **84**, 72-84 (1989) [11]

**Correlations:** Produces a three-dimensional NOE spectrum where the  $^1\text{H}$ - $^1\text{H}$  NOESY is edited through  $^{15}\text{N}$  chemical shifts. Therefore, only NOE's related to HN groups are observed. This experiment is simply a 2D NOESY experiment combined with a 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC experiment. In the diagram below, the  $^{15}\text{N}$  chemical shift is observed in the F2 dimension, the protons at the origin of the arrows are observed in the F1 dimension, and protons at the end of the arrows ( $^1\text{HN}$ ) are observed in the F3 dimension (acquisition).

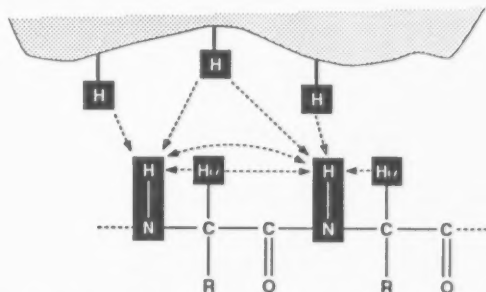


**Figure IV-17:** Correlations observed in 3D- $^{15}\text{N}$ -edited NOESYHMQC

**Coupling constant:**  $^1J_{\text{NH}} \sim 95 \text{ Hz}$

**Comments:** This experiment is used for the sequential backbone assignment, for secondary structure determination and for tertiary structure determination

*Pulse sequence:*

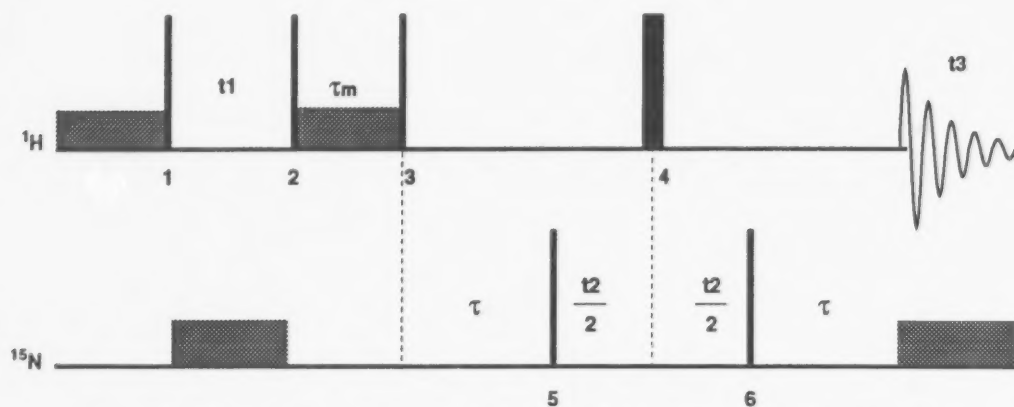


Figure IV-18: Pulse scheme of the 3D- $^{15}\text{N}$ -edited NOESYHMQC

*Phase cycling:*

|                |   |    |    |    |   |    |    |    |
|----------------|---|----|----|----|---|----|----|----|
| 1 <sup>†</sup> | x | -x | x  | -x | y | -y | y  | -y |
| 2              | x | x  | x  | x  | y | y  | y  | y  |
| 3              | x | x  | x  | x  | y | y  | y  | y  |
| 4              | x | x  | x  | x  | y | y  | y  | y  |
| 5 <sup>‡</sup> | x | x  | -x | -x | y | y  | -y | -y |
| 6              | x | x  | x  | x  | y | y  | y  | y  |
| Acq            | x | -x | -x | x  | y | -y | -y | y  |

*Delays:*

$$\tau = 1/(2 \times J_{\text{HN}})$$

$$\tau_m = 150 \text{ ms (NOESY mixing time)}$$

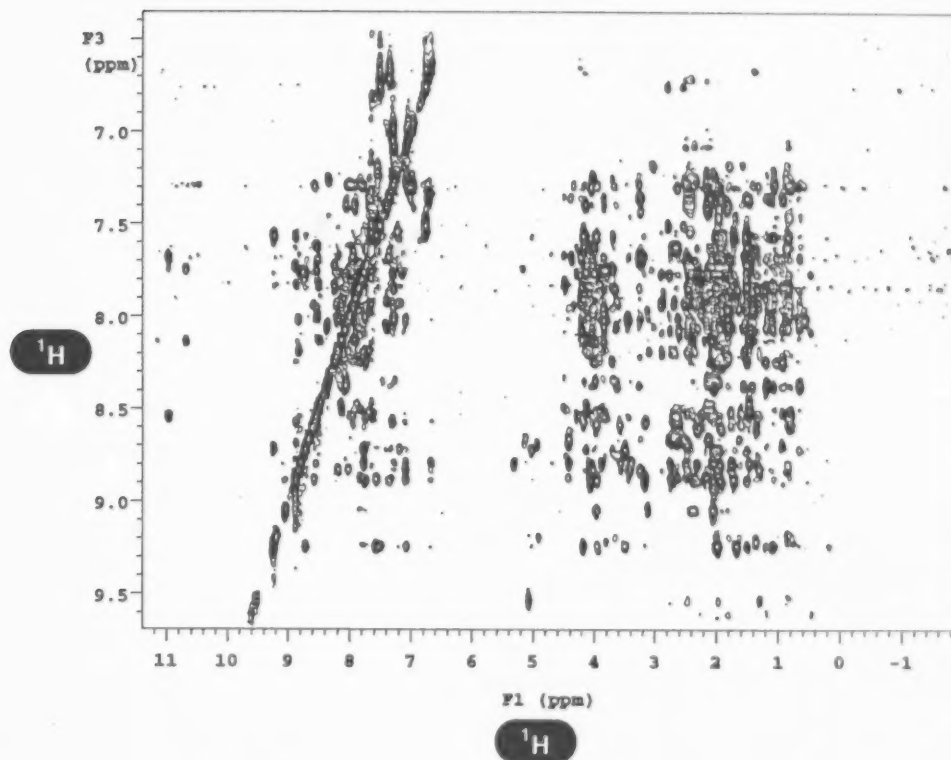


Figure IV-19. Projection of the 64 planes of the  $^{15}\text{N}$ -edited NOESYHMQC spectrum of NTnC·2Ca.

### 3D- $^{15}\text{N}$ -edited-TOCSYHMQC

**Reference:** Marion, D., Driscoll, P.C., Kay, L.E., Wingfield, P.T., Bax, A., Gronenborn, A.M. & Clore, G.M.  
*Biochemistry* **28**, 6150-6156 (1989) [12]

**Correlations:** Produces a three-dimensional TOCSY spectrum where the  $^1\text{H}$ - $^1\text{H}$  TOCSY is edited through the  $^{15}\text{N}$  chemical shift. Therefore, only TOCSY correlations related to HN groups are observed. This experiment is simply a 2D  $^1\text{H}$ - $^1\text{H}$  TOCSY experiment combined with a 2D- $\{^{15}\text{N}, ^1\text{H}\}$ -HMQC experiment. The  $^{15}\text{N}$  chemical shift is observed in the F2 dimension, the  $^1\text{HN}$  chemical shift is observed in the F3 dimension (acquisition), and all other  $^1\text{H}$ 's are observed in the F1 dimension.

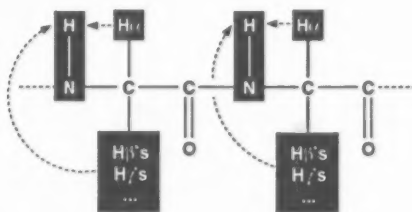
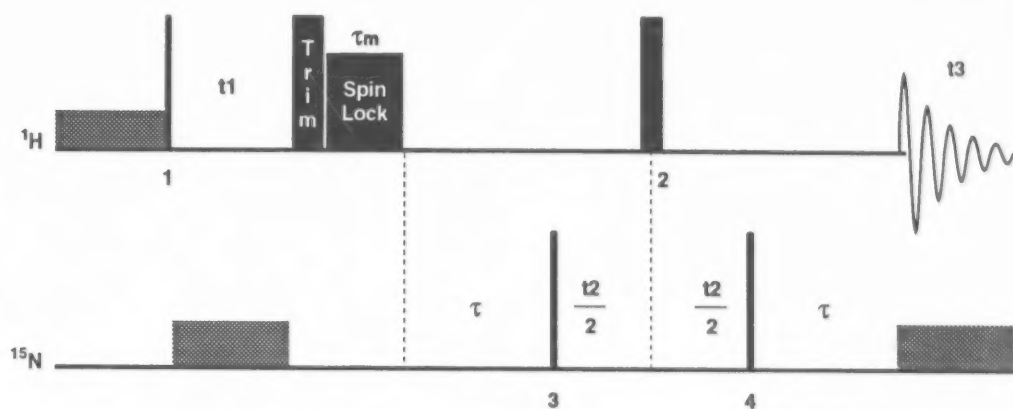


**Figure IV-20:** Correlations observed in 3D- $^{15}\text{N}$ -edited TOCSYHMQC

**Coupling constant:**  $^1J_{\text{NH}} \sim 95 \text{ Hz}$

**Comments:** This experiment is use for general assignment purpose.

*Pulse sequence:*Figure IV-21: Pulse scheme of the 3D-<sup>15</sup>N-edited TOCSYHMQC*Phase cycling:*

|                |   |    |   |    |    |    |    |    |
|----------------|---|----|---|----|----|----|----|----|
| 1 <sup>†</sup> | x | x  | y | y  |    |    |    |    |
| 2              | x | x  | y | y  |    |    |    |    |
| 3 <sup>‡</sup> | x | -x | y | -y |    |    |    |    |
| 4              | x | -x | y | -y |    |    |    |    |
| Acq            | x | x  | y | y  | -x | -x | -y | -y |

*Delays:*

$$\tau \approx 1/(2 \times {}^1J_{\text{HN}})$$

$$\tau_{\text{m}} = 70 \text{ ms (TOCSY mixing time)}$$

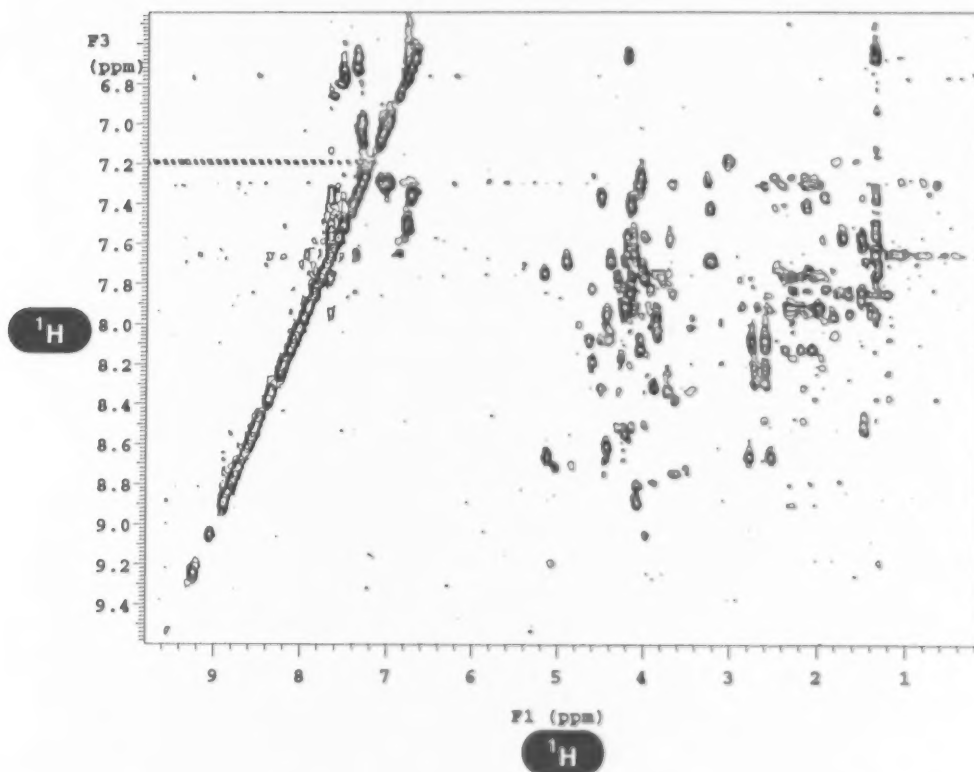


Figure IV-22. Projection of the 64 planes of the  $^{15}\text{N}$ -edited TOCSYHMQC spectrum of NTnC·2Ca.



## References

- 1 Wüthrich, K. (1986). *NMR of proteins and nucleic acids*. New York: John Wiley & Sons.
- 2 Clore, G.M. & Gronenborn, A.M. (1991). *Science* **252**, 1390-1399.
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- 8 Bax, A., Griffey, R.H. & Hawkins, B.L. (1983). *J. Magn. Reson.* **55**, 301-315.
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- 10 Powers, R., Gronenborn, A.M., Clore, G.M. & Bax, A. (1991). *J. Magn. Reson.* **94**, 209-213.
- 11 Kay, L.E., Marion, D. & Bax, A. (1989). *J. Magn. Res.* **84**, 72-84.
- 12 Marion, D., Driscoll, P.C., Kay, L.E., Wingfield, P.T., Bax, A., Gronenborn, A.M. & Clore, G.M. (1989). *Biochemistry* **28**, 6150-6156.
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- 
- 15 Summers, M.F., Marzilli, L.G. & Bax, A. (1986). *J. Amer. Chem. Soc.* **108**, 4285-4294.
  - 16 Bax, A., Griffey, R.H. & Hawkins, B.L. (1983). *J. Magn. Reson.* **55**, 301-315.
  - 17 Patt, S.L. (1992). *J. Magn. Reson.* **96**, 94-102.
  - 18 Kay, L.E., Bax, A. (1990). *J. Magn. Reson.* **86**, 110-126.
  - 19 Goodgame, M.M., Geer, S.M. (1993). *J. Magn. Reson.* **Series A** **102**, 246-248.

## CHAPTER V

# BACKBONE ASSIGNMENTS AND CALCIUM-INDUCED SECONDARY STRUCTURAL CHANGES

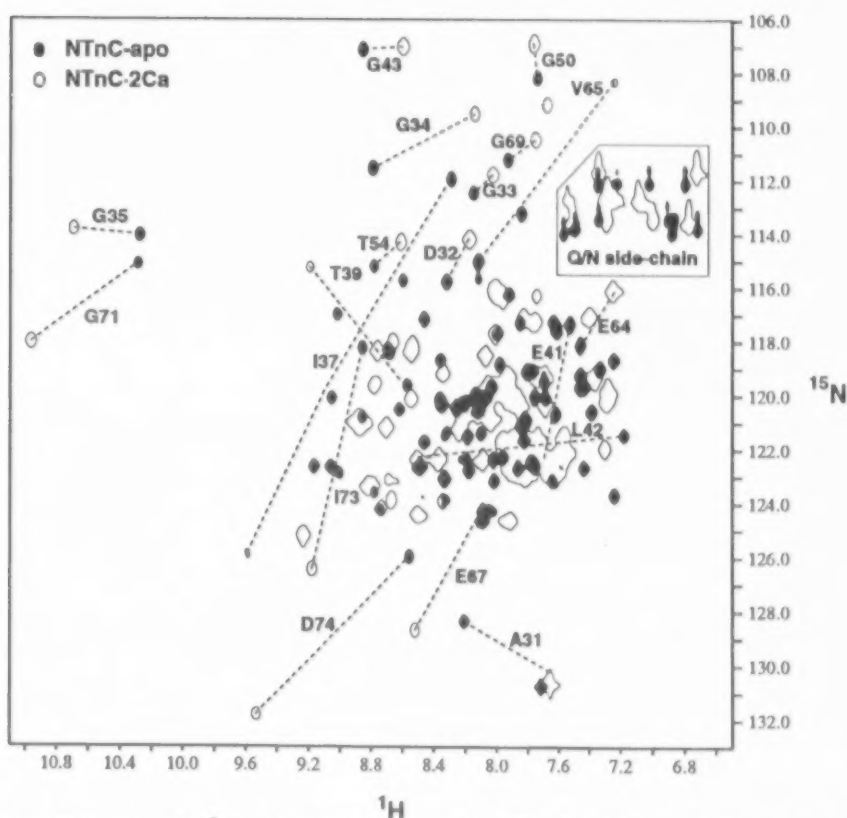


Figure V-1. Does  $\text{Ca}^{2+}$  induce any changes in NTnC? Well... a picture is worth a thousand words...

*Two- and three-dimensional multinuclear NMR provide high resolution spectra with well-resolved signals from individual nuclei that can be used to determine structure and probe conformational changes in detail. The occurrence of conformational changes in the regulatory domain of TnC is clearly indicated by the chemical shift differences between the apo and 2 Ca<sup>2+</sup> states of NTnC. These changes are very well pictured when comparing the 2D(<sup>15</sup>N-<sup>1</sup>H) HMQC spectrum of NTnC-apo and NTnC·2Ca (Fig. V-1). With the exception of the asparagine and glutamine side-chain correlations, each cross-peak in the displayed region of the 2D(<sup>15</sup>N-<sup>1</sup>H)-HMQC represents a backbone amide NH. NTnC contains 1 proline, and thus a maximum of 89 <sup>15</sup>N-<sup>1</sup>HN backbone amide correlations may be observed; 86 and 84 backbone amides were observed and assigned for NTnC-apo and NTnC·2Ca, respectively. The missing correlations correspond to amide hydrogens that are in fast exchange with the solvent, and therefore are saturated during solvent presaturation. The very low overlap between the <sup>15</sup>N and/or <sup>1</sup>H chemical shifts of NTnC-apo and NTnC·2Ca reveals that the perturbations due to Ca<sup>2+</sup> binding are propagated throughout the entire molecule. In order to properly attribute these changes to individual amino acids or regions in the protein, we first carefully assigned the backbone <sup>1</sup>HN, <sup>15</sup>N and <sup>1</sup>Hα resonances for NTnC-apo and the <sup>1</sup>HN, <sup>15</sup>N, <sup>1</sup>Hα, <sup>13</sup>Cα and <sup>13</sup>CO resonances for NTnC·2Ca.*

## Backbone assignment

### *Assignment of NTnC-apo*

The assignment for NTnC-apo has been done mainly by Sakae Tsuda and was based on conventional methods using 2D-COSY, 2D-TOCSY and 3D- $^{15}\text{N}$ -edited TOCSY experiments to identify spin-systems, and 2D-NOESY and 3D- $^{15}\text{N}$ -edited NOESY experiments to identify inter-residue connectivities along the polypeptide chain [1]. The assignment of the NTnC-apo was based in part on the assignment of the TR<sub>1</sub>C fragment [2] and followed a similar procedure except for the fact that the  $^{15}\text{N}$ -edited NOESY and TOCSY experiments were used to resolve some ambiguities. The complete  $^1\text{HN}$ ,  $^{15}\text{N}$  and  $^1\text{H}\alpha$  assignments for NTnC-apo are listed in table V-1.

### *Assignment of NTnC-2Ca*

For NTnC-2Ca a different approach, which relies on heteronuclear scalar coupling, was used to obtain sequential connectivities along the protein backbone [3]. Similar approaches which also rely on various combinations of correlations between  $^1\text{HN}$ ,  $^{15}\text{N}$ ,  $^1\text{H}\alpha$ ,  $^{13}\text{C}\alpha$  and  $^{13}\text{CO}$  nuclei have been successfully applied for various proteins, including the homologous protein calmodulin [3, 4]. Two types of sequential assignment were used: one centered on the  $^{13}\text{C}\alpha$  and another centered on the  $^{13}\text{CO}$ . The first type of sequential assignment was accomplished by combining the strong intra-residue  $^1\text{HN}(i)$ - $^{15}\text{N}(i)$ - $^{13}\text{C}\alpha(i)$  and the weaker sequential  $^1\text{HN}(i)$ - $^{15}\text{N}(i)$ - $^{13}\text{C}\alpha(i-1)$  connectivities from the HNCA experiment, the sequential  $^1\text{HN}(i)$ - $^{15}\text{N}(i)$ - $^{13}\text{C}\alpha(i-1)$  connectivity from the HNCOA experiment, and the  $d_{\text{NN}}(i, i\pm 1)$  NOE connectivities from the  $^{15}\text{N}$ -edited NOESY experiment. The role of the four chemical shift coordinates of each  $d_{\text{NN}}$  [ $^1\text{HN}(i)$ ;  $^{15}\text{N}(i)$ ;  $^{15}\text{N}(i\pm 1)$ ;  $^1\text{HN}(i\pm 1)$ ], which were automatically found in

the peak list using the in-house program CHAINS (Boyko & Gagné, MRC group in Protein Structure and Function, University of Alberta, unpublished), was to confirm the  $\text{C}\alpha$ -based sequential assignment and to resolve some ambiguities resulting from overlapping  $\text{C}\alpha$ . An example of HNCA, HNCOCA and  $d_{\text{NN}}$  connectivities is shown on figure V-2a, b and e, respectively. A total of 153 connectivities were found in the HNCA (80 intra-residue and 73 sequential) and 77 sequential connectivities in the HNCOCA. The second strategy for sequential assignment, now relying on the  $^{13}\text{C}\text{O}$ , was performed by combining the intra-residue  $^1\text{H}\alpha(i)\text{-}^{13}\text{C}\alpha(i)\text{-}^{13}\text{C}\text{O}(i)$  connectivity from the HCACO experiment, the sequential  $^1\text{HN}(i+1)\text{-}^{15}\text{N}(i+1)\text{-}^{13}\text{C}\text{O}(i)$  connectivity from the HNCO experiment, and the intra-residue  $^1\text{HN}(i)\text{-}^{15}\text{N}(i)\text{-}^1\text{H}\alpha(i)$  from the  $^{15}\text{N}$ -edited TOCSY experiment. An example of these connectivities is shown on figure V-2c, d and f. Overall, 89 intra-residue connectivities were found in the HCACO, and 82 sequential ones in the HNCO. In both procedures, the  $^{15}\text{N}$ -edited TOCSY was used to identify the spin-system related to each  $^1\text{HN}/^{15}\text{N}$  pair. Not all spin-systems could be identified in the TOCSY experiment, mainly because of the small  $^1\text{HN}\text{-}^1\text{H}\alpha$  coupling constants which are characteristic of  $\alpha$ -helices. However, residue identification could often be helped by comparing the observed  $^1\text{H}\alpha$ ,  $^{13}\text{C}\alpha$  and  $^{13}\text{C}\text{O}$  chemical shifts to their corresponding expected value [5]. Using this procedure, the near-complete  $^1\text{HN}$ ,  $^{15}\text{N}$ ,  $^1\text{H}\alpha$ ,  $^{13}\text{C}\alpha$  and  $^{13}\text{C}\text{O}$  resonance assignments of NTnC-2Ca have been achieved and listed in Table V-2.

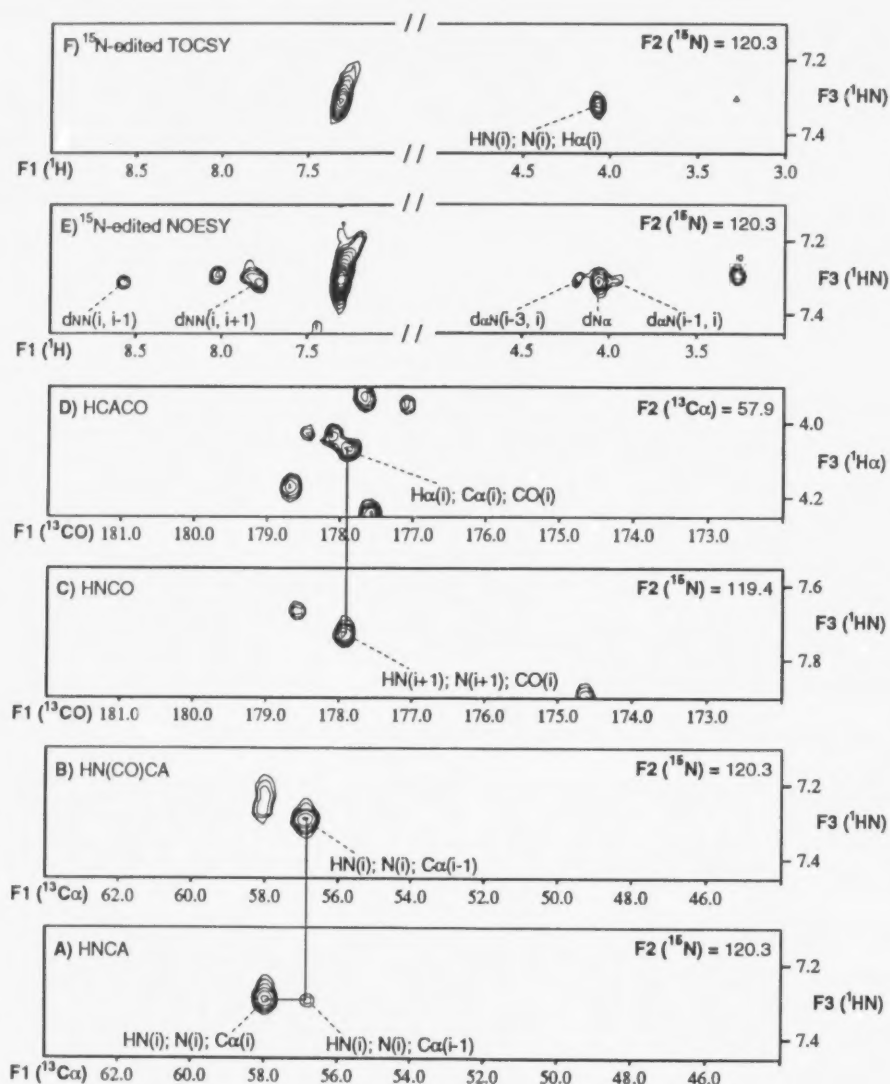


**Figure V-2.** Connectivities related to one residue of NTnC-2Ca, M28, in various 3D-experiments. The HNCA (A) and HNCOCA (B) experiments provide sequential information via the  $^{13}\text{C}\alpha$  chemical shift. Similar sequential information can also be obtained via the  $^{13}\text{CO}$  chemical shift by combining the HNCO (C), the HCACO (D) and the  $^{15}\text{N}$ -edited TOCSY (F) experiments. The  $^{15}\text{N}$ -edited NOESY (E) experiment also provide sequential information [d $_{\text{NN}}$ , d $_{\alpha\text{N}}$ (i-3, i) and d $_{\alpha\text{N}}$ (i-1, i)].



Table V-1. Polypeptide Backbone  $^1\text{H}$  and  $^{15}\text{N}$  Chemical Shifts for the apo N-Domain of Chicken Troponin-C at pH6.6 and 30 °C

|     | $^{15}\text{N}$ | $^1\text{HN}$ | $^1\text{H}\alpha$ |     | $^{15}\text{N}$ | $^1\text{HN}$ | $^1\text{H}\alpha$ |     | $^{15}\text{N}$ | $^1\text{HN}$ | $^1\text{H}\alpha$ |
|-----|-----------------|---------------|--------------------|-----|-----------------|---------------|--------------------|-----|-----------------|---------------|--------------------|
| A1  | —               | —             | 4.12               | A31 | 128.4           | 8.15          | 4.10               | I61 | 122.3           | 7.94          | 3.75               |
| S2  | —               | —             | 4.45               | D32 | 115.8           | 8.33          | 4.72               | I62 | 121.8           | 8.45          | 3.45               |
| M3  | —               | —             | 4.60               | G33 | 112.6           | 8.13          | 3.79, 4.05         | E63 | 119.1           | 7.76          | 3.99               |
| T4  | 115.7           | 8.12          | 4.22               | G34 | 111.6           | 8.78          | 3.92, 4.16         | E64 | 118.1           | 7.46          | 4.13               |
| D5  | 121.6           | 8.34          | 4.50               | G35 | 114.0           | 10.28         | 3.80, 4.34         | V65 | 115.0           | 8.11          | 4.21               |
| O6  | 121.5           | 8.29          | 4.14               | D36 | 119.1           | 7.81          | 5.58               | D66 | 123.1           | 8.34          | 5.15               |
| Q7  | 122.3           | 8.18          | 4.00               | I37 | 111.9           | 8.31          | 4.65               | E67 | 124.3           | 8.07          | 4.12               |
| A8  | 124.3           | 8.05          | 4.13               | S38 | 115.8           | 8.58          | 4.86               | D68 | 117.2           | 8.45          | 4.66               |
| E9  | 120.6           | 8.12          | 4.07               | T39 | 119.6           | 8.58          | 3.76               | G69 | 111.3           | 7.93          | 3.85, 3.91         |
| A10 | 124.8           | 8.10          | 4.13               | K40 | 121.4           | 8.10          | 4.14               | S70 | 120.1           | 9.05          | 4.26               |
| R11 | 116.4           | 7.93          | 3.90               | E41 | 117.3           | 7.53          | 4.70               | G71 | 115.1           | 10.28         | 3.86, 4.16         |
| A12 | 120.8           | 7.61          | 4.25               | L42 | 121.4           | 7.19          | 3.77               | T72 | 113.3           | 7.88          | 5.43               |
| F13 | 119.4           | 7.69          | 4.35               | G43 | 107.1           | 8.84          | 3.49, 3.76         | I73 | 118.3           | 8.87          | 4.79               |
| L14 | 119.8           | 7.42          | 4.38               | T44 | 119.8           | 7.45          | 3.76               | D74 | 126.0           | 8.57          | 5.61               |
| S15 | 117.3           | 7.86          | 4.50               | V45 | 123.2           | 7.63          | 3.54               | F75 | 120.1           | 8.36          | 3.50               |
| E16 | 122.9           | 9.00          | 3.91               | M46 | 118.7           | 8.35          | 3.81               | E76 | 117.8           | 7.96          | 3.75               |
| E17 | 120.8           | 8.87          | 4.05               | R47 | 120.2           | 8.12          | 4.61               | E76 | 120.5           | 8.27          | 3.97               |
| M18 | 122.7           | 7.75          | 3.99               | M48 | 123.2           | 8.01          | 4.23               | F78 | 122.7           | 8.46          | 3.98               |
| I19 | 120.7           | 8.09          | 3.37               | L49 | 119.0           | 7.33          | 4.57               | L79 | 120.2           | 8.11          | 3.42               |
| A20 | 121.1           | 7.83          | 4.17               | G50 | 108.2           | 7.74          | 3.81, 4.30         | V80 | 118.8           | 7.25          | 3.28               |
| E21 | 122.8           | 7.85          | 4.17               | Q51 | 119.6           | 8.03          | 4.46               | M81 | 119.9           | 7.75          | 3.91               |
| F22 | 122.7           | 9.04          | 4.90               | N52 | 118.5           | 8.69          | 5.15               | M82 | 120.3           | 8.18          | 4.04               |
| K23 | 122.7           | 9.17          | 3.75               | P53 | —               | —             | 4.75               | V83 | 121.5           | 8.19          | 3.55               |
| A24 | 121.7           | 7.84          | 4.17               | T54 | 115.1           | 8.79          | 4.35               | R84 | 120.2           | 7.68          | 3.87               |
| A25 | 120.8           | 7.38          | 4.32               | K55 | 123.7           | 8.78          | 3.91               | Q85 | 117.7           | 7.59          | 3.98               |
| F26 | 120.3           | 8.35          | 3.66               | E56 | 118.3           | 8.75          | 4.05               | M86 | 118.8           | 7.93          | 4.20               |
| D27 | 116.8           | 9.02          | 4.33               | E57 | 122.4           | 7.79          | 4.01               | K87 | 120.9           | 7.79          | 4.16               |
| M28 | 119.3           | 7.45          | 3.94               | L58 | 122.6           | 8.50          | 4.02               | E88 | 122.3           | 8.10          | 4.26               |
| F29 | 117.3           | 7.64          | 4.34               | D59 | 120.6           | 8.63          | 4.32               | D89 | 122.8           | 8.20          | 4.63               |
| D30 | 123.7           | 7.24          | 4.87               | A60 | 122.7           | 7.42          | 4.19               | A90 | 130.8           | 7.70          | 4.12               |

Table V-2: Polypeptide Backbone  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$  Chemical Shifts for the  $\text{Ca}^{2+}$ -saturated state of the N-Domain of Chicken Troponin-C at pH6.7 and 30 °C

|     | $^{15}\text{N}$ | $^{13}\text{C}\alpha$ | $^{13}\text{CO}$ | $^1\text{HN}$ | $^1\text{H}\alpha$ |     | $^{15}\text{N}$ | $^{13}\text{C}\alpha$ | $^{13}\text{CO}$ | $^1\text{HN}$ | $^1\text{H}\alpha$ |
|-----|-----------------|-----------------------|------------------|---------------|--------------------|-----|-----------------|-----------------------|------------------|---------------|--------------------|
| A1  | —               | 51.8                  | 174.2            | —             | 4.14               | M46 | 117.7           | 59.4                  | 178.8            | 8.01          | 3.91               |
| S2  | —               | 58.3                  | 173.9            | —             | 4.52               | R47 | 120.5           | 58.9                  | 181.0            | 8.22          | 4.64               |
| M3  | —               | 56.0                  | 176.6            | —             | 4.64               | M48 | 122.8           | 58.8                  | 175.6            | 7.94          | 4.22               |
| T4  | 116.1           | 61.1                  | 175.0            | 8.01          | 4.44               | L49 | —               | 53.6                  | 176.9            | 7.19          | 4.34               |
| D5  | 123.0           | 57.3                  | 178.7            | 8.69          | 4.44               | G50 | 106.9           | 45.7                  | 174.6            | 7.75          | 3.70, 4.12         |
| Q6  | 120.9           | 58.8                  | 179.1            | 8.87          | 4.07               | Q51 | 119.4           | 53.9                  | 174.3            | 7.95          | 4.43               |
| Q7  | 123.0           | 58.3                  | 177.8            | 7.83          | 3.93               | N52 | 118.0           | 51.0                  | 172.1            | 8.66          | 5.14               |
| A8  | 124.4           | 54.9                  | 181.1            | 8.50          | 4.14               | P53 | —               | 62.6                  | 177.8            | —             | 4.74               |
| E9  | 120.5           | 58.8                  | 178.8            | 8.13          | 4.04               | T54 | 114.3           | 60.2                  | 175.2            | 8.60          | 4.43               |
| A10 | 124.5           | 55.5                  | 179.2            | 7.92          | 4.11               | K55 | 123.3           | 60.0                  | 178.3            | 8.79          | 3.88               |
| R11 | 116.5           | 59.4                  | 178.0            | 7.96          | 3.85               | E56 | 118.3           | 60.6                  | 180.1            | 8.80          | 4.08               |
| A12 | 120.4           | 53.7                  | 178.6            | 7.69          | 4.23               | E57 | 122.6           | 60.4                  | 179.5            | 7.77          | 4.00               |
| F13 | 119.4           | 59.2                  | 175.7            | 7.68          | 4.38               | L58 | 121.6           | 58.0                  | 178.6            | 7.96          | 4.03               |
| L14 | 119.4           | 53.6                  | 176.0            | 7.37          | 4.50               | D59 | 120.1           | 57.2                  | 179.0            | 8.54          | 4.30               |
| S15 | 117.2           | 56.3                  | 175.0            | 7.83          | 4.59               | A60 | 123.0           | 54.8                  | 180.3            | 7.63          | 4.18               |
| E16 | 122.7           | 60.1                  | 179.6            | 9.05          | 3.97               | I61 | 121.5           | 65.0                  | 178.3            | 7.57          | 3.71               |
| E17 | 120.9           | 60.2                  | 179.1            | 8.89          | 4.07               | I62 | 119.3           | 65.0                  | 177.7            | 7.46          | 3.42               |
| M18 | 121.6           | 58.3                  | 178.1            | 7.73          | 4.03               | E63 | 119.1           | 59.3                  | 178.2            | 8.35          | 4.00               |
| I19 | 121.2           | 66.8                  | 177.6            | 8.24          | 3.72               | E64 | 116.2           | 58.9                  | 179.4            | 7.27          | 4.04               |
| A20 | 122.2           | 55.1                  | 181.3            | 7.84          | 4.19               | V65 | 108.3           | 60.8                  | 175.4            | 7.26          | 4.59               |
| E21 | 122.2           | 59.1                  | 180.3            | 7.77          | 4.14               | D66 | —               | 53.5                  | 177.3            | —             | 4.70               |
| F22 | 121.2           | 58.8                  | 178.5            | 8.72          | 5.03               | E67 | 128.7           | 59.2                  | 176.9            | 8.53          | 4.23               |
| K23 | 125.1           | 58.3                  | 177.2            | 9.24          | 3.95               | D68 | 116.2           | 52.4                  | 177.7            | 8.01          | 4.73               |
| A24 | 122.2           | 54.9                  | 180.7            | 7.57          | 4.19               | G69 | 110.4           | 47.1                  | 175.3            | 7.75          | 3.81, 3.88         |
| A25 | 122.0           | 55.0                  | 177.9            | 7.57          | 3.98               | S70 | 118.2           | 60.1                  | 176.2            | 8.54          | 4.22               |
| F26 | 121.2           | 62.2                  | 176.6            | 8.88          | 3.17               | G71 | 118.0           | 45.6                  | 172.8            | 10.97         | 3.44, 4.13         |
| D27 | 118.6           | 56.9                  | 177.8            | 8.57          | 4.19               | T72 | 109.3           | 58.1                  | 173.5            | 7.68          | 4.92               |
| M28 | 120.3           | 57.9                  | 177.9            | 7.30          | 4.05               | I73 | 126.5           | 60.4                  | 176.2            | 9.19          | 5.07               |
| F29 | 119.4           | 58.2                  | 177.7            | 7.78          | 4.30               | D74 | 131.9           | 53.1                  | 175.9            | 9.53          | 5.32               |
| D30 | 119.7           | 52.1                  | 176.7            | 8.08          | 4.50               | F75 | 119.7           | 61.5                  | 176.4            | 8.79          | 3.59               |
| A31 | 130.4           | 55.0                  | 179.1            | 7.68          | 4.11               | E76 | 117.3           | 58.8                  | 180.3            | 7.77          | 3.80               |
| D32 | 114.3           | 52.7                  | 177.8            | 8.19          | 4.59               | E76 | 120.7           | 57.9                  | 178.8            | 8.19          | 4.17               |
| G33 | 111.8           | 46.7                  | 175.7            | 8.02          | 3.85, 3.85         | F78 | 123.4           | 61.2                  | 177.3            | 8.83          | 4.04               |
| G34 | 109.6           | 46.3                  | 175.9            | 8.14          | 3.98, 4.09         | L79 | 119.8           | 58.0                  | 179.1            | 8.02          | 3.47               |
| G35 | 113.8           | 45.3                  | 173.2            | 10.69         | 3.67, 4.47         | V80 | 120.3           | 67.2                  | 178.2            | 7.29          | 3.27               |
| D36 | 116.2           | 52.6                  | 173.1            | 7.75          | 5.17               | M81 | 121.5           | 59.3                  | 178.2            | 7.85          | 3.69               |
| I37 | 125.9           | 60.1                  | 175.7            | 9.61          | 4.94               | M82 | 118.6           | 56.4                  | 179.4            | 8.07          | 4.05               |
| S38 | 124.0           | 55.7                  | 175.9            | 8.70          | 4.85               | V83 | 122.3           | 66.9                  | 178.1            | 8.38          | 3.64               |
| T39 | 115.1           | 66.9                  | 177.0            | 9.22          | 3.82               | R84 | 120.8           | 59.4                  | 179.0            | 8.06          | 3.86               |
| K40 | 122.7           | 59.3                  | 179.7            | 7.84          | 4.12               | Q85 | 117.2           | 57.3                  | 177.9            | 7.40          | 4.15               |
| E41 | 122.3           | 58.8                  | 179.7            | 7.70          | 4.13               | M86 | 120.1           | 57.9                  | 177.6            | 7.92          | 4.25               |
| L42 | 122.4           | 57.8                  | 178.7            | 8.52          | 3.99               | K87 | 120.4           | 57.5                  | 177.1            | 7.86          | 4.17               |
| G43 | 107.1           | 47.8                  | 175.5            | 8.58          | 3.59, 3.98         | E88 | 121.9           | 57.0                  | 176.5            | 7.91          | 4.30               |
| T44 | 119.7           | 67.2                  | 176.3            | 7.94          | 3.94               | D89 | 122.3           | 54.0                  | 174.9            | 8.08          | 4.63               |
| V45 | 122.0           | 66.3                  | 177.5            | 7.30          | 3.69               | A90 | 130.7           | 53.7                  | 170.6            | 7.65          | 4.12               |

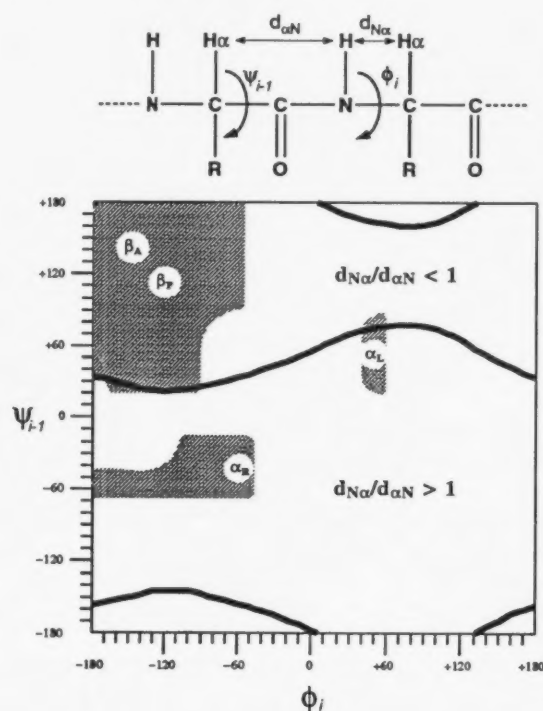
## Secondary structure

### *Secondary structure determination strategy*

There are three reliable secondary structure determination methods that are commonly used in NMR: one is based on the NOE connectivities characteristic of different secondary structures [1], another is based on the  $^3J_{\text{HNH}\alpha}$  coupling constants [12], and the last one is based on the characteristic backbone chemical shift values of the  $\alpha$ -helix,  $\beta$ -sheet and random coil residues [5, 6]. I have applied the three approaches to NTnC-apo and NTnC-2Ca, with special attention to the quantification aspects of the NOE approach.

NOE connectivities found in NOESY spectra usually contain, among others, the  $d_{\alpha\text{N}}(i-1, i)$  and the  $d_{\text{N}\alpha}(i, i)$ . The  $d_{\text{N}\alpha}(i, i)$  NOE is non-informative by itself, the intra-residue HN-H $\alpha$  distance being covalently restricted to between 2.2 and 3.1 Å, and being realistically found in the 2.7 to 3.1 Å range for  $\alpha$ -helices and  $\beta$ -sheets. For the  $d_{\alpha\text{N}}(i-1, i)$  NOE, the allowed HN<sub>(i)</sub>-H $\alpha$ <sub>(i-1)</sub> distance is found in a wider range (2.2 to 3.6 Å), and the magnitude of the  $d_{\alpha\text{N}}(i-1, i)$  NOE could be used to differentiate between a residue found in the right-handed  $\alpha$ -helix region ( $3.4 \text{ \AA} < \text{HN}_{(i)}\text{-H}\alpha_{(i-1)} < 3.6 \text{ \AA}$ ) and one in the  $\beta$ -sheet region ( $2.2 \text{ \AA} < \text{HN}_{(i)}\text{-H}\alpha_{(i-1)} < 3.1 \text{ \AA}$ ). However, the inaccuracy of this measurement (mainly due to the variation of amide exchange rate along the sequence) makes it a poor criterion for secondary structure identification. This inadequacy can be overcome by using the ratio of these two NOE's,  $d_{\text{N}\alpha}/d_{\alpha\text{N}}$  (for simplicity,  $d_{\text{N}\alpha}(i, i)$  NOE/ $d_{\alpha\text{N}}(i-1, i)$  NOE is abbreviated to  $d_{\text{N}\alpha}/d_{\alpha\text{N}}$ ). This ratio is larger than 1 for  $\beta$ -strands, and smaller than 1 for right-handed  $\alpha$ -helices (see figure V-3). Since these two NOE's are related to the same amide HN, the  $d_{\text{N}\alpha}/d_{\alpha\text{N}}$  ratio has the advantage of being independent of amide exchange and can be quantitated more safely. Note that the magnitude of this ratio relates mainly to the  $\psi$  torsion angle of residue (i-1), if only the right-handed  $\alpha$ -helix and the

**Figure V-3.**  $\phi/\psi$  map showing the relation between the  $d_{N\alpha}/d_{\alpha N}$  ratio and the major secondary structure regions. The shaded areas represent the energy-favoured  $\phi_i/\psi_{i-1}$  regions for two residues in a protein.  $\beta_A$ ,  $\beta_P$ ,  $\alpha_R$  and  $\alpha_L$  represent ideal  $\phi/\psi$  angle for antiparallel  $\beta$ -sheet, parallel  $\beta$ -sheet, right-handed  $\alpha$ -helix and left-handed  $\alpha$ -helix, respectively. The thick line is the contour for  $d_{N\alpha}/d_{\alpha N} = 1$  from a contour map generated by varying the  $\phi_2$  and  $\psi_1$  in a dipeptide.  $d_{N\alpha}/d_{\alpha N}$  represent the expected ratio between the  $d_{N\alpha}(i, i)$  and  $d_{\alpha N}(i-1, i)$  NOEs obtained from the measure of  $[r(\text{HN}_i\text{-H}\alpha_{i-1}) / r(\text{HN}_i\text{-H}\alpha_i)]^{1/6}$ , where  $r$  is the distance between two protons. A representation of the various parameters used in the  $\phi/\psi$  map for a two residues is shown above the map. The  $\beta$ -sheet region is clearly characterized by a  $d_{N\alpha}/d_{\alpha N} < 1$  whereas the right-handed  $\alpha$ -helix is localized in the  $d_{N\alpha}/d_{\alpha N} > 1$  region, making the  $d_{N\alpha}/d_{\alpha N}$  ratio an accurate criteria for secondary structure determination.



$\beta$ -sheet regions are considered. We therefore used this ratio as one of the criteria for secondary structure characterization, by measuring the ratio of the intensity of the  $d_{\alpha N}(i-1, i)$  and  $d_{N\alpha}(i, i)$  NOE found on the  $^{15}\text{N}$  plane of residue (i) in the  $^{15}\text{N}$ -edited NOESY. The second NOE criterion used is the  $d_{\alpha N}(i-3, i)$  connectivity which is representative of  $\alpha$ -helices [1]. Examples of  $d_{N\alpha}$ ,  $d_{\alpha N}$  and  $d_{\alpha N}(i-3, i)$  connectivities can be found in figure V-2e. The third criterion included in the determination of the secondary structure is the chemical shift index, which is applied using the methodology described by Wishart & Sykes [5, 6]. The CSI was determined using the  $^1\text{H}\alpha$  chemical shifts for NTnC-apo, and the consensus of the  $^1\text{H}\alpha$ ,  $^{13}\text{C}\alpha$  and

$^{13}\text{C}$ O indexes without any smoothing for NTnC-2Ca. Structural information about torsion angles can also be obtained from scalar coupling constants based on Karplus equations [13]. The relation between the backbone  $\phi$  angle and the  $^3J_{\text{HNH}\alpha}$  coupling constant is available [12] and is used as the fourth criterion to defined secondary structure.  $^3J_{\text{HNH}\alpha} < 6\text{Hz}$  was taken to indicate helices, whereas  $^3J_{\text{HNH}\alpha} > 8\text{Hz}$  was taken to indicate  $\beta$  secondary structure. Unambiguous absence of splitting at high resolution enhancement (LB = -28) was interpreted as  $^3J_{\text{HNH}\alpha} < 6\text{Hz}$ .

Definition of helices can be a delicate issue, since different methods can be used [7]. Therefore it is vital to clearly stipulate the approach used before stating that an helix starts or ends at a certain residue. The information obtained from the data presented in this paper is the following: (1) the occurrence of a  $d_{\text{N}\alpha}/d_{\alpha\text{N}} > 1$  indicates that residue  $i-1$  possesses a  $\psi$  angle in the  $\alpha$ -helical region; (2)  $d_{\alpha\text{N}}(i-3, i)$  NOE suggest that residue  $i-3$ ,  $i-2$ , and  $i-1$  are in helical conformation, but does not give any information about the  $\phi/\psi$  angle of residue  $i$ , although it may suggest that  $\text{HN}_i$  is hydrogen-bonded to either  $\text{CO}_{i-4}$  or  $\text{CO}_{i-3}$ ; (3) the CSI reports predominantly information about the  $\phi$  and  $\psi$  angle of its related residue, since backbone chemical shifts are closely related to the main-chain  $\phi/\psi$  angle [8]; (4) the  $^3J_{\text{HNH}\alpha}$  reports estimation of the  $\phi$  angle. Ideally all four criteria should agree, but due to factors which are not related to secondary structure (NOE overlap, spin-diffusion, chemical shift affected by aromatic ring, etc.), a consensus is used instead. Therefore, helices were defined using the following rules: (1) residues were defined as helical when more than half of the available criteria (consensus of  $d_{\text{N}\alpha}/d_{\alpha\text{N}}$ ,  $d_{\alpha\text{N}}(i-3, i)$ , CSI, and  $^3J_{\text{HNH}\alpha}$ ) were characteristic of an  $\alpha$ -helix; (2) at least 4 consecutive helical residues were necessary to start a helix; (3) the N-terminal residue of a helix must possess a consensus of the following: a  $d_{\text{N}\alpha}/d_{\alpha\text{N}} > 1$  with the next residue, a  $d_{\alpha\text{N}}(i-3, i)$ , an  $\alpha$ -helix CSI and a  $^3J_{\text{HNH}\alpha} < 6\text{Hz}$ ; (4) the C-terminus of a helix must have a consensus of the following: a  $d_{\text{N}\alpha}/d_{\alpha\text{N}} > 1$  with the next residue, a  $d_{\alpha\text{N}}(i-3, i)$  between residue  $i-2$  and  $i+1$ , an  $\alpha$ -helix



CSI, and a  $^3J_{\text{HNH}\alpha} < 6$  Hz. The methodology used in this study for secondary structure determination is therefore closely related to a secondary structure definition based on  $\phi/\psi$  angle [7]. The compilation of the  $d_{\text{N}\alpha}/d_{\alpha\text{N}}$ ,  $d_{\alpha\text{N}}(\text{i-3, i})$ , CSI and  $^3J_{\text{HNH}\alpha}$ , along with the secondary structure, for NTnC-apo and NTnC-2Ca are represented in figure V-4 and V-5, respectively.

*NTnC-apo; secondary structure*

Five helices were found by NMR for NTnC-apo: helix N, A, B, C and D. This is the same as is found in the crystal structures of TnC. According to the criteria described above, the N-helix is well defined from T4 to F13. The tight turn linking the N-helix and the A-helix is characterized by a  $d_{\text{N}\alpha}/d_{\alpha\text{N}} < 1$  observed for S15 and E16, indicating positive  $\psi$  angles for both L14 and S15. The A-helix unambiguously spans from E16 to M28. The negative CSI of F22 in the center of the A-helix might occur due to a deshielding orientation of the aromatic ring. No regular secondary structure is observed for the F29-G35 segment, whereas the next three residues are well defined as  $\beta$ -strand by the CSI. The ( $d_{\text{N}\alpha}/d_{\alpha\text{N}} < 1$ ) between I37 and T39 indicate positive  $\psi$  angles between D36 and S38, and the  $^3J_{\text{HNH}\alpha} > 8\text{Hz}$  of D36 and I37 indicate  $\beta$ -strand characteristic  $\phi$  angle for these residues.

The B-helix of NTnC-apo is less well defined by the NOE's due to some  $^1\text{H}\alpha$  resonances appearing very close to the water resonance (4.70 and 4.61 for E41 and R47, respectively) and  $^1\text{H}\alpha$  overlap (in particular in the L42-T44 segment). Even if some of the  $d_{\text{N}\alpha}/d_{\alpha\text{N}}$  and  $d_{\alpha\text{N}}(\text{i-3, i})$  are ambiguous in the B-helix, the absence of an NOE between K40- $\text{H}\alpha$  and G43-HN is unambiguous (figure V-6). This is in perfect agreement with the crystal structure, where these two protons are separated by 5.19Å. The  $^3J_{\text{HNH}\alpha} > 8\text{Hz}$  of E41 is also in agreement with the X-ray coordinates, where this residue had a  $\phi$  angle of  $-96^\circ$ . The T39-M48 stretch shows two  $\beta$ -sheet-like CSI; one for E41, and the other for R47. These two non-helical

chemical shifts are however consistent with the geometry of the B-helix in the crystal structure, as demonstrated by chemical shift calculations made using the crystal coordinates and the SHIFTS program (Jellard et al., PENCE, University of Alberta; Ösapay & Case, 1991). These calculations showed  $\alpha$ -helix-like upfield shift relative to the random coil value for all  $^1\text{H}\alpha$  in the T39-M48 segment, except for E41 due to irregular  $\phi/\psi$  angles ( $-96^\circ/-7^\circ$ ), and for K47 due to the particular orientation of the Q51 C=O bond. The rationalization related to K47H $\alpha$  is based on chemical shift calculations with and without Q51CO, which demonstrated that Q51CO induces a downfield shift on K47H $\alpha$ . Helix-B therefore spans from L42 to M48, but can also be viewed as spanning from T39 to M48 with a kink at residue E41.

No particular secondary structure is associated with L49-T54. The last two helices, C and D, are very well defined, spanning from K55 to E64 and F75 to K87, respectively. The second  $\text{Ca}^{2+}$ -binding loop, like the first one, possesses no distinct NOE or CSI features for the first six residues, and a  $\beta$ -strand for the last three residues, T72-D74. The three C-terminal residues are relatively flexible/unstructured





**Figure V-4.** Schematic representation of the secondary structure determination of NTnC-apo.  $d_{\alpha\text{N}}(i-3, i)$  represents the NOE connectivity between  $\text{H}\alpha_{i-3}$  and  $\text{HN}_i$ ;  $d_{\text{N}\alpha}/d_{\alpha\text{N}}$  represents the ratio between the  $d_{\text{N}\alpha}(i, i)$  and  $d_{\alpha\text{N}}(i-1, i)$  NOE intensities (upward and downward relate to  $d_{\text{N}\alpha}/d_{\alpha\text{N}} > 1$  and  $d_{\text{N}\alpha}/d_{\alpha\text{N}} < 1$ , respectively); CSI represents the chemical shift index (Wishart & Sykes, 1994a, 1994b) for the  $\alpha$ -protons only (upward and downward relate to  $\alpha$ -helix and  $\beta$ -strand characteristic, respectively);  $^3J_{\text{HN-H}\alpha}$  reports values of  $J < 6\text{ Hz}$  ( $\alpha$ ),  $J > 8\text{ Hz}$  ( $\beta$ ). Intermediate  $J$ 's are represented by a dash (-), and ambiguous or unavailable  $J$ 's by a question mark (?). Dotted lines and open squares indicate ambiguities. The compilation of the secondary structure as determined by these criteria is found on the last line of each segments; rectangles and arrows indicate helices and  $\beta$ -strand, respectively. The kink in the B-helix (see text) is represented by a break at residue 41.

*NTnC·2Ca; secondary structure*

Five helices are also found in the calcium saturated form of NTnC, with slightly different lengths for some of them in comparison to the apo form. Considering that the amide protons of the first 3 residues are not observed due to fast exchange, and that the CSI is variable for the A1-T4 segment, the first four residues are probably disordered. The N-helix is very well defined from D5 to F13. The A-helix is also well delineated from E16 to F29 using our criteria, having only one ambiguous  $d_{\alpha N}(i-3, i)$ . The first calcium-binding loop of NTnC does not show any regular secondary structure features from D30 to G35. The binding-loop ends with a short  $\beta$ -strand (D36-S38) defined by negative CSI's for D36, I37 and S38,  $d_{N\alpha}/d_{\alpha N} < 1$  for I37 and S38, and  $^3J_{\text{HNH}\alpha} > 8$  Hz for I37 and S38. The B-helix is the least-well defined using the  $d_{N\alpha}/d_{\alpha N}$  criterion, due to overlap or very weak NOEs. However, the  $d_{\alpha N}(i-3, i)$ , CSI and  $^3J_{\text{HNH}\alpha}$  unambiguously characterize the B-helix from T39 to R47. Unfortunately, since the  $^{15}\text{N}$  of L49 is unassigned, the only secondary structure information available for M48 is its CSI and its  $^3J_{\text{HNH}\alpha}$ , which are both characteristic of helices. Helix-B is therefore assumed to extend from T39 to M48. The L49-T54 section of NTnC·2Ca shows variations for both the  $d_{N\alpha}/d_{\alpha N}$  and the CSI, and is categorized as a 6 residue linker between helices B and C. The C-helix is also well characterized from K55 to E63, with some ambiguity about its C-terminus. As can be seen in the 2D( $^{15}\text{N}$ - $^1\text{H}$ ) HMQC NMR spectrum (figure V-1), V65 is one of the weakest observable amides, leading to virtually no observable NOEs in the 3D- $^{15}\text{N}$ -edited NOESY. In addition, the amide of D66 is unassigned or non-observable, so that only the CSI and the  $^3J_{\text{HNH}\alpha}$  are available to characterize E64. The C-helix therefore ended at E64. The V65-G71 segment does not have any  $\alpha$ -helix or  $\beta$ -sheet character. Finally, the second  $\beta$ -strand (T72-D74) and helix-D (F75-K87) are well defined by the NMR data. The C-terminal residues E88-A90 are flexible, as for the apo structure.



Figure V-5. Schematic representation of the secondary structure determination of NTnC-2Ca. The representation used is the same as in figure V-4, except for the CSI, which is a consensus of the  $^1\text{H}\alpha$ ,  $^{13}\text{C}\alpha$  and  $^{13}\text{CO}$  index.

## Ca<sup>2+</sup>-induced conformational changes

### Secondary structural change

In order to fully characterize the secondary structural changes that occur in NTnC upon calcium binding, we compared the secondary structures obtained for NTnC-apo and NTnC-2Ca from this study, the NMR secondary structure of the TR<sub>1</sub>C fragment [2], and the crystal structure of TnC [10]. To obtain a comparison as consistent as possible for the helices, all structures were compared in terms of "NMR helices". For the X-ray structure, we extracted the  $d_{N\alpha}/d_{\alpha N}$  ratio NOE, the  $d_{\alpha N}(i-3, i)$  NOE and the  $^3J_{HNH\alpha}$  expected from the crystal coordinates based on distance alone or on  $\phi$  angle, and used them to define the helices with the approach described above for NTnC. The TR<sub>1</sub>C helices were defined based on the NOE data presented by [2]. The comparison of the helices from these four structures is summarized in Table V-3. The N-helix can be considered identical for the

Table V-3: Location of  $\alpha$ -Helices in the N-Domain of Troponin-C

| $\alpha$ -helix | Residue Range            |                                      |                       |                       |
|-----------------|--------------------------|--------------------------------------|-----------------------|-----------------------|
|                 | X-ray (apo) <sup>a</sup> | TR <sub>1</sub> C (apo) <sup>b</sup> | NTnC-apo <sup>c</sup> | NTnC-Ca2 <sup>c</sup> |
| N               | M3-F13                   | —                                    | T4-F13                | D5-F13                |
| A               | E16-M28                  | E16-D26 <sup>d</sup>                 | E16-M28               | E16-F29               |
| B               | T39-K40                  | L42-L49                              | T39-K40               | T39-M48               |
|                 | L42-M48                  |                                      | L42-M48               |                       |
| C               | K55-E64                  | K55-E64                              | K55-E64               | K55-E64               |
| D               | F75-A90                  | F75-M86                              | F75-R87               | F75-R87               |

<sup>a</sup> Herzberg & James (1988). Helix limits based on expected  $^3J_{HNH\alpha}$  and NOE's from crystal coordinates.

<sup>b</sup> Findlay & Sykes (1993). NMR secondary structure of turkey skeletal TnC fragment (residues 12-87).

<sup>c</sup> Present study. Helix range based on  $^3J_{HNH\alpha}$ , CSI,  $d_{\alpha N}(i, i+3)$  NOE and  $d_{N\alpha}/d_{\alpha N}$  NOE ratio.

<sup>d</sup> Revision of the NOE data of TR<sub>1</sub>C suggest that the C-termini of helix-A is M28 (see text)

x-ray, NTnC-apo and NTnC·2Ca, the only possible variance being different flexibility for the first few residues. The A-helix is invariant between the x-ray and NTnC-apo, and is one residue longer in NTnC·2Ca. Although the NOE's presented previously for TR<sub>1</sub>C indicated D26 as being the C-terminus of helix-A, the chemical shift identities between NTnC-apo and TR<sub>1</sub>C for the <sup>1</sup>H $\alpha$  of residues E16-A31 ( $\Delta\delta \leq 0.02$  ppm) suggests that both have identical  $\phi/\psi$  angles. A revision of the NOE data related to helix-A of TR<sub>1</sub>C (data not shown) showed NOE connectivities identical to the ones presented in this paper for NTnC-apo, and therefore helix-A is believed to end at M28 in all 3 apo structures and at F29 in the Ca<sup>2+</sup> structure.

The B-helix in the crystal structure possesses a kink due to a deviation from ideal helical  $\phi/\psi$  angles at E41 ( $\phi=-96^\circ$ ,  $\psi=-7^\circ$ ). This  $\phi$  angle is reflected in the NTnC-apo solution structure by a <sup>3</sup>J<sub>HNH $\alpha$</sub>  > 8 Hz (figure 7). In terms of expected d <sub>$\alpha$ N</sub>(i-3, i) in the crystal structure, there should be a strong NOE for L42 (3.0 Å), a non-observable NOE for G43 (5.2 Å), and medium NOE's for the T44-G50 stretch (3.3-3.8 Å). The NTnC-apo NOESY spectrum shows an ambiguous d <sub>$\alpha$ N</sub>(i-3, i) for L42 and none for G43, whereas the NTnC·2Ca spectrum reveals d <sub>$\alpha$ N</sub>(i-3, i) for both L42 and G43 (figure V-6). Consequently, the kink at the N-terminus of helix-B is observed in the x-ray, TR<sub>1</sub>C and NTnC-apo structures. This kink is however absent in the structure of NTnC·2Ca, as indicated by the NOE connectivities (figure V-5 and V-6) and <sup>3</sup>J<sub>HNH $\alpha$</sub>  < 6Hz (figure 5 and 7).

Site II of NTnC-apo is similar to the crystal conformation, as indicated by the G69 d <sub>$\alpha$ N</sub>(i-3, i) NOE (fig. V-4). This NOE is the only d <sub>$\alpha$ N</sub>(i-3, i) expected for a non-helical region in the crystal structure (3.69 Å). The NOE spectrum of NTnC·2Ca does not exhibit this d <sub>$\alpha$ N</sub>(i-3, i), indicating some Ca<sup>2+</sup>-induced conformational change in site II. Finally, the conformation of helix-D is not affected by Ca<sup>2+</sup>-binding.

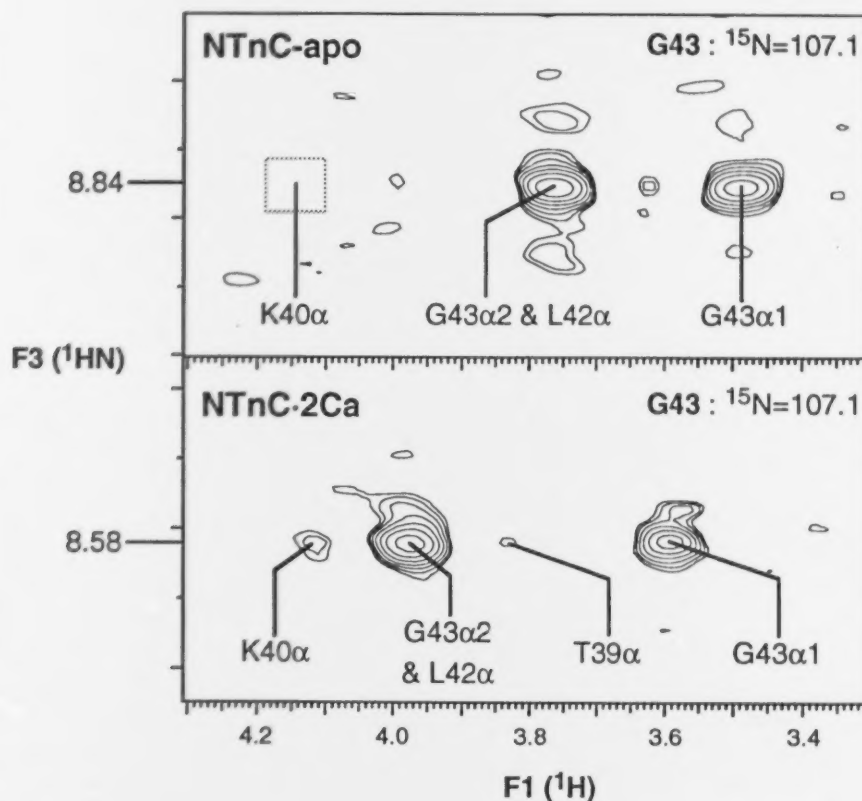


**Figure V-6.** Comparison of the NOE connectivities of G43's amide proton for NTnC-apo and NTnC-2Ca in their  $^{15}\text{N}$ -edited NOESY. The 3D NOESY spectrum of NTnC-2Ca unambiguously indicates the presence of a  $d_{\alpha\text{N}}(\text{i-3}, \text{i})$  between K40 and G43. This NOE is absent in the spectrum of NTnC-apo, as would be expected from the crystal structure (see text); the expected position of this  $d_{\alpha\text{N}}(\text{i-3}, \text{i})$  is indicated by a box. Note that the  $^{15}\text{N}$ -edited NOESY spectra of NTnC-apo is plotted at the noise level. This difference in NOE connectivity between NTnC-apo and NTnC-2Ca is one of the indications that the kink of helix-B at E41 is straightened upon  $\text{Ca}^{2+}$ -binding. The observation of a very weak  $d_{\alpha\text{N}}(\text{i-4}, \text{i})$  between T39 and G43 in the spectra of NTnC-2Ca also support this idea (those two protons are separated by 5.39 Å in the crystal structure of TnC).



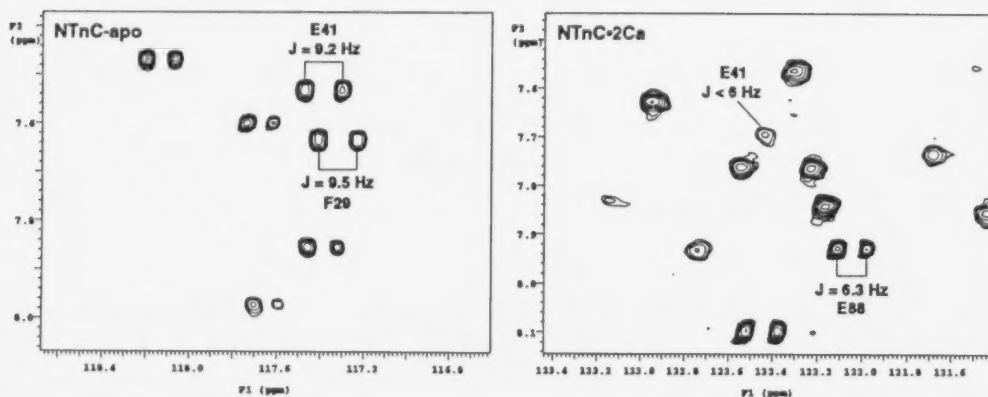


**Figure V-7.** Comparison of the  $^3J_{\text{HNH}\alpha}$  coupling constant of E41 for NTnC-apo and NTnC-2Ca in their HMQC-J spectrum. A coupling constant of 9.2 Hz was measured for NTnC-apo using the HMQCJFIT program (see text), indicating that E41 has a  $\beta$  characteristic  $\phi$  angle in the apo form of NTnC. In the spectrum of NTnC-2Ca, no splitting is observed, indicating that E41 has a  $^3J_{\text{HNH}\alpha} < 6$  Hz and that this residue has an  $\alpha$  characteristic  $\phi$  angle in the  $\text{Ca}^{2+}$  form of NTnC. Note the splitting observed for E88 ( $^3J_{\text{HNH}\alpha} = 6.3$  Hz) which clearly indicate that E41 has a  $^3J_{\text{HNH}\alpha} < 6$  Hz in NTnC-2Ca. Both spectrum were processed in the same way (VNMR parameters GF=0.05, LB=-28).



*Chemical shift change*

Since the chemical shift of a nucleus is affected by its environment, the chemical shift changes which occur in NTnC upon  $\text{Ca}^{2+}$ -binding can be interpreted in terms of structural changes. Figure V-8 compiles those changes for each residue, using a relative chemical shift change which includes  $^1\text{H}\alpha$ ,  $^1\text{HN}$  and  $^{15}\text{N}$ . As expected, site I and II are the most affected by the binding of  $\text{Ca}^{2+}$  due to conformational changes and the proximate presence of two calcium ions. The most affected residue is I37 (figure V-1 and V-8), located in the middle of the first  $\beta$ -strand. The second  $\beta$ -strand chemical shifts are also strongly affected. Interestingly, the largest variation observed outside of the binding loops occurs at E41 and L42; this observation is consistent with the NOE variation observed at the beginning of the B-helix (figure V-6). On average, the B/A helix pair is strongly affected by the calcium-induced structural change, whereas the C-D pair is less perturbed. The N-helix is only weakly perturbed, as is the B-C linker.

*Helical content*

In order to assess the helical content of NTnC-apo and NTnC-2Ca, I used two approaches, one based upon number of residues in helical segments, and a second based upon all residues susceptible of having helical  $\phi/\psi$  angles. I first directly count the number of residues found in helices according to the data presented in figure V-4 and V-5 for the two forms of NTnC. The NMR secondary structure of NTnC-apo shows 54-56 residues in well-defined helices, and a similar number, 55, is found for NTnC-2Ca. Even if this helical residue count is not perfectly accurate, these results do not explain the  $\text{Ca}^{2+}$ -induced ellipticity increase observed in the CD spectra of NTnC [11].

The second approach attempts to estimate the proportion of time that each residue spends with helical  $\phi/\psi$  angles. Due to the relationships

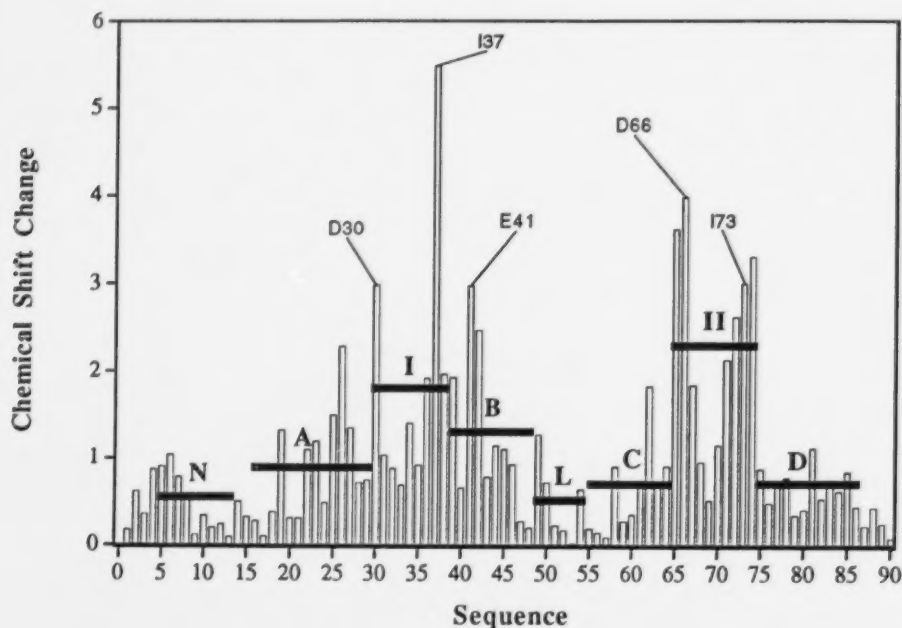


**Figure V-8.** Chemical shift change along the sequence of NTnC induced by the binding of calcium. The chemical shift change for each residue is obtained by averaging the normalized change of the backbone  $^1\text{H}\alpha$ ,  $^1\text{HN}$  and  $^{15}\text{N}$  chemical shifts. The normalized chemical shift change for a particular nucleus of each residue is obtained by dividing the observed shift change by the average shift change for all residues. Therefore, a chemical shift change of 1 is equivalent to the average change observed for all residues. The horizontal bars indicated the average change for various segments of NTnC: N, A, B, C and D represent the five respective helices, I and II represent the binding loop in site I and II, and L represents the B-C linker. As expected, the largest changes are observed in the two  $\text{Ca}^{2+}$ -binding loops. The third largest change is found in helix-B, mainly due to E41 and L42; this observation is one of the evidences for the straightening of that helix upon  $\text{Ca}^{2+}$ -binding.

between  $\alpha$ -proton chemical shifts and backbone dihedral angles [8], the helical content of a protein (in terms of  $\phi$ ,  $\psi$ ) can also be approximated by using the chemical shift differences ( $\Delta\delta$ ) from the random coil value [5]. In this case, every residue with an upfield shift relative to the random coil

value is accounted for, whether it is in a helix or not. By its nature, this method is quite comparable to the quantification of secondary structure made by CD. We calculated the average  $\Delta\delta$ 's from the random coil values for the  $^1\text{H}\alpha$ 's of NTnC-apo and NTnC-2Ca. 62 residues had an upfield shift characteristic of  $\alpha$ -helical structure in NTnC-apo, with an average  $\Delta\delta$  of -0.37 ppm. The NTnC-2Ca assignment revealed 63 upfield-shifted  $^1\text{H}\alpha$ , with an average  $\Delta\delta$  of -0.36 ppm. The regular shift from random-coil chemical shift in helical residues is -0.38 ppm [5]. The crystal structure of TnC has 65 residues with  $\psi$  between  $-10^\circ$  and  $-70^\circ$ . The number of upfield-shifted  $\alpha$ -protons for NTnC-apo and NTnC-2Ca is in agreement with the crystal structure, especially when considering that truncation at residue 90 in NTnC is likely to reduce helix-D by 2-3 residues compared to the whole TnC. As for the results from the residue count, the  $\Delta\delta$ 's do not show any increase of helical content in NTnC upon  $\text{Ca}^{2+}$ -binding.

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## CHAPTER VI

SIDE-CHAIN ASSIGNMENTS AND  
PRELIMINARY TERTIARY  
STRUCTURE

*The previous chapter reported a detailed quantification of the calcium-induced secondary structural changes in the regulatory domain of troponin-C. This quantification clearly shows that the secondary structural features of the N-domain of TnC are not significantly affected by the binding of  $\text{Ca}^{2+}$ . Now that the secondary structure question has been answered, others must be asked. "What are the calcium-induced tertiary structural changes?". "Is the model proposed by Herzberg et al. [1] close to reality?".*

*This chapter presents results that answer these questions. The results involved are preliminary and need more refinement. Therefore this chapter is kept to a minimum as its outcome, the very low resolution solution structure of NTnC·2Ca, is regarded only as a "Note added in proof" to the discussion made in the next chapter.*

## Getting distance restraints

### *Side-chain assignments*

The assignment of the side-chain  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts has been achieved using the following experiments:  $^{15}\text{N}$ -edited TOCSYHMQC,  $^{15}\text{N}$ -edited NOESYHMQC, HCCHCOSY [4], and  $^{13}\text{C}$ -edited NOESYHMQC. The  $^{15}\text{N}$ -edited TOCSYHMQC and the  $^{15}\text{N}$ -edited NOESYHMQC experiments have been described in chapter IV. The  $^{13}\text{C}$ -edited NOESYHMQC was not described in previous chapters, but is basically the same as the  $^{15}\text{N}$ -edited NOESYHMQC, except for the fact that it is  $^{13}\text{C}$ -edited. The HCCHCOSY [4] pulse sequence was kindly provided by Lewis E. Kay (Univ. of Toronto) and is a  $^{13}\text{C}$ -edited through-bond 3D-experiment correlating two protons having their carbon separated by one bond. A schematic representation of the correlation expected in each of these five experiments is shown in figure VI-1 for a glutamate residue. Although all four experiments were necessary to complete the backbone assignment, the HCCHCOSY was definitely the most useful one. Examples of the two  $^{13}\text{C}$ -edited experiments used for this assignment are shown in figure VI-2 and VI-3. Examples of some planes of the two  $^{15}\text{N}$ -edited experiments were given in chapter V.

The side-chain assignments have been completed at approximately 90 %. The missing assignments are usually due to serious overlap. A list of the chemical shifts assigned so far for NTnC·2Ca can be found in appendix B.



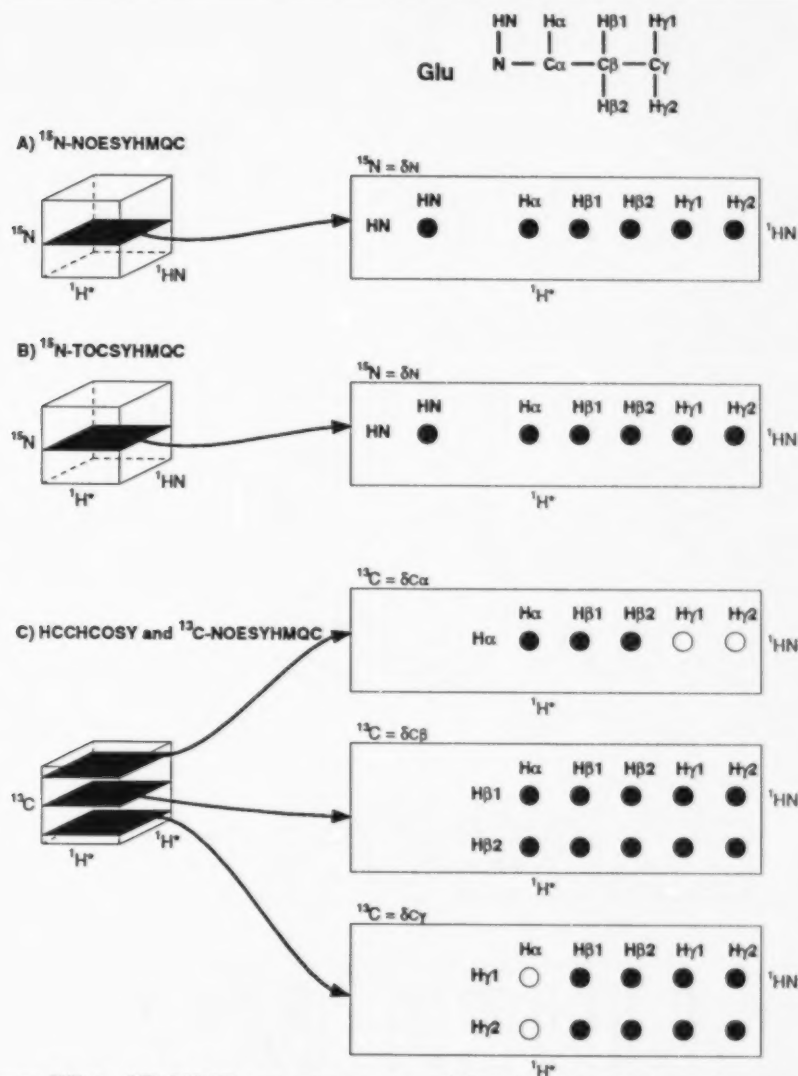


**Figure VI-1.** 3D-NMR experiments used for the side-chain assignments of NTnC-2Ca. **A)**  $^{15}\text{N}$ -edited NOESYHMQC usually show NOE cross-peak between backbone amide and every other intra-residue protons (also show a number of inter-residue cross-peak). **B)**  $^{15}\text{N}$ -edited TOCSYHMQC can show correlation between amide and all intra-residue proton, but often (especially with helical proteins) correlation with protons further than the H $\beta$ 's are not observed. **C)** HCCHCOSY and  $^{13}\text{C}$ -edited NOESYHMQC are two  $^{13}\text{C}$ -edited experiments. Filled peaks represent correlations expected in both experiments and opened peaks represent correlations observed only in the  $^{13}\text{C}$ -edited NOESYHMQC. As for the  $^{15}\text{N}$ -edited NOESY, the  $^{13}\text{C}$ -edited NOESY also shows a number of inter-residue correlations (not shown here).



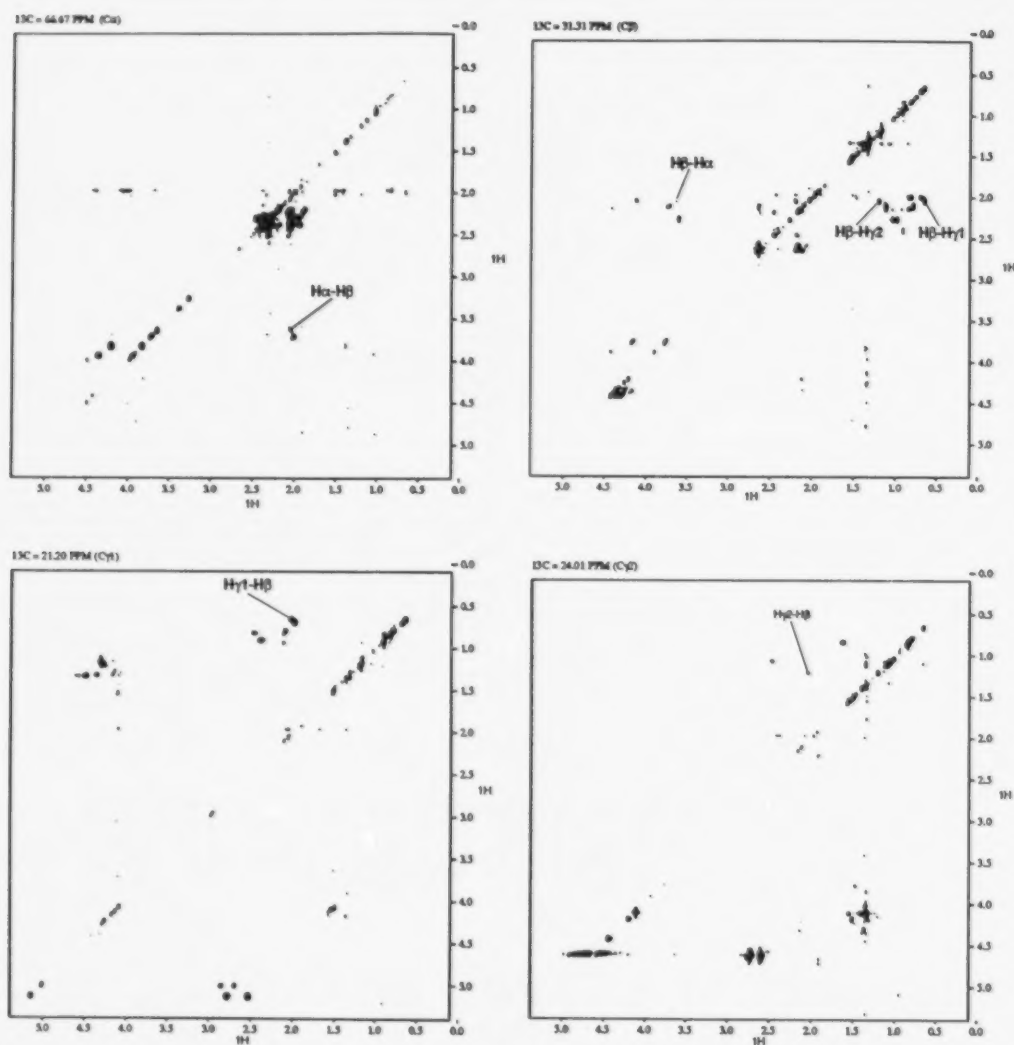


**Figure VI-2.** Correlations found for Val83 in the HCHCOSY spectrum. Valine residues having four aliphatic carbons, four sets of correlations can be found on four  $^{13}\text{C}$  planes corresponding to the  $^{13}\text{C}$  chemical shifts of valine's C $\alpha$ , C $\beta$ , C $\gamma$ 1 and C $\gamma$ 2.

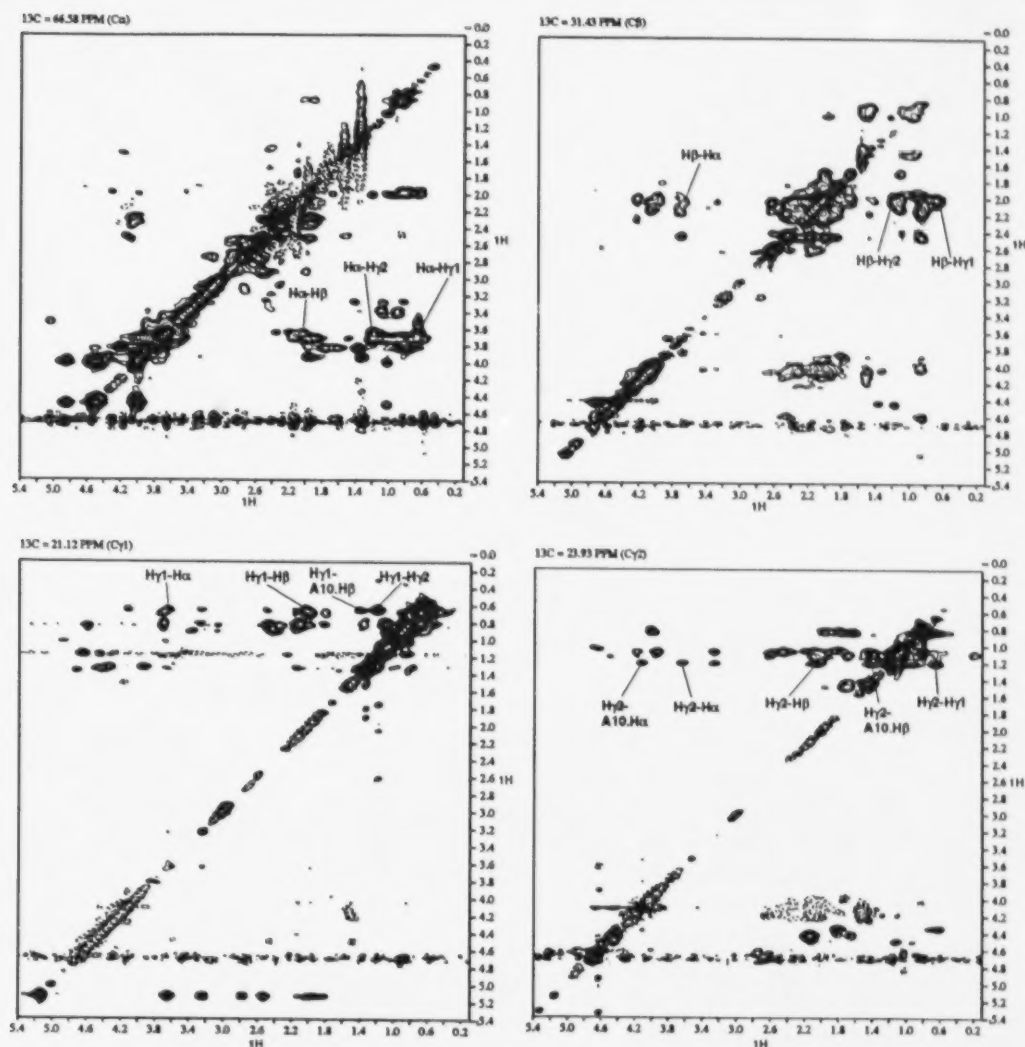


Figure VI-3. Correlations found for Val83 in the  $^{13}\text{C}$ -edited NOESYHMQC spectrum. As for the HCCHCOSY, correlations can be found on four different planes corresponding to the  $\text{C}\alpha$ ,  $\text{C}\beta$ ,  $\text{C}\gamma 1$  and  $\text{C}\gamma 2$  chemical shifts. Note the few long range NOE's between a N-terminal residues (Ala10) and C-terminal residue (Val83)

*NOE assignments*

The assignment of the NOE's in the  $^{15}\text{N}$ -edited NOESYHMQC and  $^{13}\text{C}$ -edited NOESYHMQC spectra was performed with the PIPP software [2]. The PIPP program takes as input a chemical shift list, a NOESY spectra and optionally one or more pdb coordinate files. The program works as follows: 1) PIPP displays the spectra; 2) the user clicks on an NOE peak; 3) PIPP compares the chemical shift coordinates of the peak selected with the chemical shifts assignment list and selects possible assignments within a specified error range; 4) if pdb coordinates are provided, it does a second "trim" by keeping only the assignments that are in agreement within a specified distance boundary with the structure(s) provided; 5) from the assignments proposed, the user can then manually choose an assignment. The specified chemical shift error range was  $\pm 0.04$  ppm for  $^1\text{H}$  and  $\pm 0.75$  ppm for  $^{13}\text{C}$  and  $^{15}\text{N}$ . Two sets of coordinates were provided to the program: one was the crystal structure coordinates and the other the model coordinates[1]. The distance cutoff was set to 12 Å, small enough to discard a few unrealistic assignment possibilities, but large enough not to bias the NOE assignments. Any assignment that was ambiguous was left unassigned. Using this approach, over 1000 NOE's were assigned.

*NOE calibration*

Calibration of NOE is often a tricky issue, and it is at its worse when it comes to three-dimensional NOE spectra. Immediately after the assignment stage, it was obvious that a single calibration for all the NOE's was inappropriate. This is due to factors like different  $^{13}\text{C}$  relaxation rate or different amide exchange rate among various residues.

To solve this problem, I applied the ideas I developped for the secondary structure characterization to the calibration of NOE's. As was explained in chapter V, the distances (or dihedral angles) related to  $d_{\text{N}\alpha}$  and

$d_{\alpha N}$  NOE's can be determined fairly accurately using the  $d_{N\alpha}/d_{\alpha N}$  ratio. Therefore the  $d_{\alpha N}$  NOE's were calibrated by comparison to the  $d_{N\alpha}$  NOE. Additionally, the  $d_{\alpha N}(i, i+3)$  were calibrated by comparison to both the  $d_{N\alpha}$  and the  $d_{\alpha N}$  NOE's. Note that these three NOE's are all related to the same amide and therefore appear on the same  $^{15}\text{N}$  plane. The actual calibration used for these NOE's is summarized in the little "nawk" scripts listed at the beginning of appendix C. Note that only those NOE's are calibrated, and all the others are set to an upper limit of 5 Å. The final list of all the NOE's used for structure calibration, along with their lower and upper distance limits, is found in appendix C.

## Tertiary structure

### *Structure calculation*

Using the DGII program (Biosym Technologies) and the restraints listed in appendix C, 64 structures were calculated. The parameters used for the calculations are listed at the end of appendix C.

### *Structure families*

It was obvious after just a few structure calculations that all the structures would not converge the same way. Therefore families of structures were defined in the following way:

- 1) Calculation of the pair-wise RMSD for the 64 structure (RMSD of backbone N, C $\alpha$  and CO for residues 4-87 only). Structure #15 has the largest number (15) a pair-wise RMSD < 2.0 Å.
- 2) Superimposition of the 15 structures to structure #15 and then average these 16 structures to generate an average structure (AVG\_1). The average RMSD of the 16 structures to AVG\_1 is 1.45 $\pm$ 0.17 Å.
- 3) Superimpose all 64 structures to AVG\_1. 24 structures are found to have RMSD < 2.0 Å.
- 4) Average the 24 structures (AVG\_2). The average RMSD of the 24 structures to AVG\_2 is 1.55 $\pm$ 0.22 Å.
- 5) Superimpose 64 structures to AVG\_2. 25 structures are found to have RMSD < 2.0 Å.
- 6) Average the 25 structures (AVG\_3). The average RMSD of the 25 structures to AVG\_3 is 1.56 $\pm$ 0.23 Å.
- 7) Superimpose 64 structures to AVG\_3. Still 25 structures are found to have RMSD < 2.0 Å. 28 structures have RMSD < 2.5 Å.
- 8) Average the 28 structures (AVG\_4). The average RMSD of the 28

structures to AVG\_4 is  $1.62 \pm 0.26$  Å.

9) Superimpose 64 structures to AVG\_4. 29 structures are found to have  $\text{RMSD} < 2.5$  Å. There is no additional structures if RMSD cutoff is increased to 3.0 Å.

10) Average of the 29 structures (AVG\_5). The average RMSD of the 29 structures to AVG\_5 is  $1.67 \pm 0.29$  Å.

These 29 structures are considered as being part of the same family of structures. Using the same cyclic approach, the left structures were classified in 5 additional families. The characteristic of those 6 families of structures are summarized in Table VI-1.

**Table VI-1.** Characteristic of the family of structures.

| Family      | Error Function <sup>†</sup> | NOE Violations/residue ( $> 0.1$ Å) |
|-------------|-----------------------------|-------------------------------------|
| #1 (29)     | 0.106                       | 1.5                                 |
| #2 (11)     | 0.221                       | 7.0                                 |
| #3 (5)      | 0.148                       | 3.4                                 |
| #4 (3)      | 0.175                       | 4.3                                 |
| #5 (3)      | 0.246                       | 8.7                                 |
| others (13) | 0.308                       | 10.4                                |

<sup>†</sup> The error function is a function which measures the total violations of the constraints by the coordinates.

Since family #1 is the most populated, the one with the lowest energy and the one with the less violations, it is considered as being the "good structure". Therefore only this family will be considered, and it is referred to as the tertiary structure of NTnC·2Ca.

*Structure characteristic*

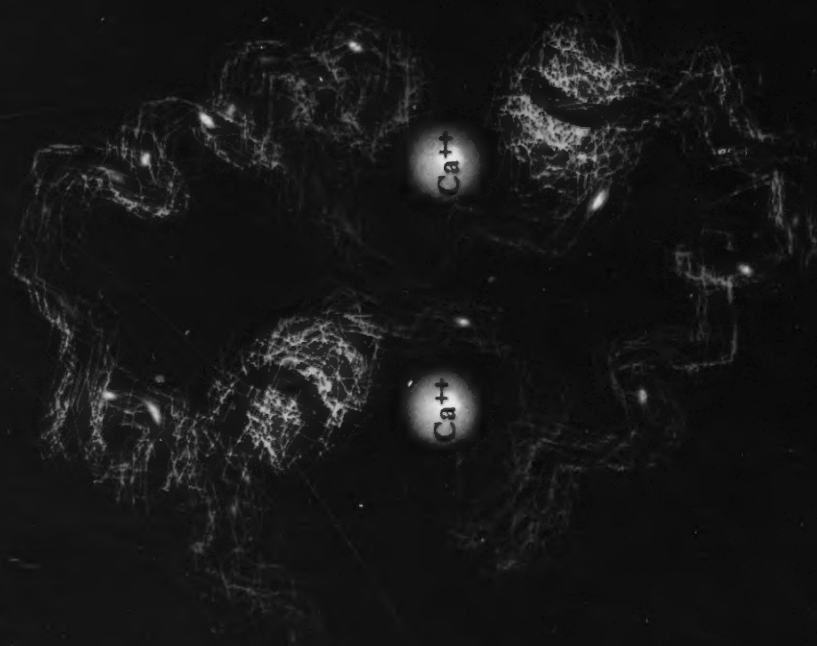
The superimposition of 29 DGII structures of NTnC·2Ca with their average are shown in Figure VI-4 along with some structure statistics. Although the resolution of the NMR structure is very low at the present stage, the global fold of the protein can be characterized. The relative orientation of the helices in this solution structure of NTnC·2Ca are quite different from the crystal structure and more similar to the proposed model. Although the present NMR structure of NTnC·2Ca shows obvious differences with the model, it is still too early to make any statements. The solution structure of NTnC-apo has also been recently solved by NMR (Sakae Tsuda et al., personal communication) and it is clear that the structure of the regulatory domain of TnC is opening upon  $\text{Ca}^{2+}$ -binding, as was predicted by Herzberg et al. [1] (figure VI-5).

**Figure VI-4.** (page 111) Solution NMR structure of the regulatory domain of TnC. 29 structures generated with DGII are superimposed to their average structure. The two  $\text{Ca}^{2+}$  ions were added and positioned by comparison with the coordinates of the C-domain of TnC in the crystal structure of  $2\text{Ca}^{2+}$  TnC [3]. The RMSD graph (top right) indicate that the two  $\text{Ca}^{2+}$ -binding loops, the B-C linker and the C-terminal part of NTnC are poorly defined. The number of NOE violations is small, as shown in the bar graph at the bottom right. Only two violations greater than 0.25 Å can be found in 29 structures.

**Figure VI-5.** (page 112) Superimposition of the apo and  $\text{Ca}^{2+}$ -saturated average NMR structure of the N-domain of TnC. The superimposition clearly shows the opening of the regulatory domain upon  $\text{Ca}^{2+}$ -binding. The opening is very similar to the model proposed by Herzberg, Moult & James [1]. Although there is some differences, the NMR structures need to be refined before conclusions may be made.

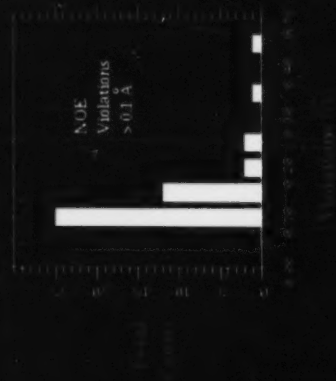


# N-Domain of TnC bound to two $\text{Ca}^{++}$ ; The NMR structure



29 NMR structures

NTnC- $\text{Ca}_2$ ; Atomic RMS distribution of 29 DGI structures



# Calcium-induced structural change of N-Domain of TnC



Superposition of helices N, A and D

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**References**

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CHAPTER **VII****DISCUSSION**

It is well recognized that muscle contraction is triggered by a  $\text{Ca}^{2+}$ -induced structural change in the regulatory domain of TnC (see review by Grabarek et al. [1]). The results presented in this paper indicate that the secondary structural features of NTnC are not strongly affected by the binding of calcium. For both NTnC-apo and NTnC·2Ca, an N-terminal helix and two helix-loop-helix motifs joined by a short  $\beta$ -sheet are observed in solution. When compared to the crystal structure of turkey TnC where the N-domain is found in the apo form [2], the NMR solution data of NTnC-apo shows only similarities. Even the kink observed by x-ray crystallography at residue 41 of helix-B is observed in solution, as supported by the absence of the G43  $d_{\alpha\text{N}}(\text{i-3, i})$  the non-helical  $^1\text{H}\alpha$  chemical shift of E41.

The secondary structure of NTnC·2Ca, as determined by NMR, is very similar to NTnC-apo (figure V-4 and V-5). The binding of calcium to NTnC does not induce any noticeable differences in terms of secondary structure for three of the five helices. Helix-C and helix-B appear to be shortened by one residue at their C-termini in NTnC·2Ca. Although some ambiguity is related to these observations, the length and relative position of helix-B and helix-C in NTnC·2Ca are exactly the same as observed in the NMR secondary structure of homologous  $\text{Ca}^{2+}$ -binding protein calmodulin [3]. Helix-A and -D were also found at the same position in calmodulin as in NTnC·2Ca, even though calmodulin lacks the N-helix.

The geometry of the B-helix is affected. The kink at residue 41 is removed upon calcium binding, leading to a regular helix from T39 to M48. These changes result in an increase of symmetry between the two binding

sites upon addition of calcium. This is in agreement with the model proposed by Herzberg et al. [4] and many helix-loop-helix protein structures which were solved in the  $\text{Ca}^{2+}$ -saturated state either by X-ray crystallography [5, 6] or NMR [7, 8]. Since the conformational changes occurring in the two  $\text{Ca}^{2+}$ -binding loops are not presented here in detail and must await the refinement of the tertiary structure of NTnC·2Ca, the straightening of helix-B is the major  $\text{Ca}^{2+}$ -induced structural change we can assess at this time. This change is localized at E41, a position which corresponds to the third most conserved residue in calcium-binding sites of proteins having the helix-loop-helix motif [9]. This invariant glutamate has both oxygen atoms of its carboxylate group coordinating the  $\text{Ca}^{2+}$  ion in a bidentate manner [5]. The E41  $\phi/\psi$  dihedral angles in the crystal structure of TnC are  $-96^\circ/-7^\circ$ , whereas the average angles for that position in 11 loops that have  $\text{Ca}^{2+}$  bound (from 5 different  $\text{Ca}^{2+}$ -binding proteins) are  $-66^\circ/-41^\circ$  [5]. The E41  $\phi/\psi$  transition from  $-96^\circ/-7^\circ$  to  $-66^\circ/-41^\circ$  can, by itself, account for most of the  $\text{Ca}^{2+}$ -induced reorientation of the B- and C-helix described by the Herzberg model [4] and observed by NMR in the preliminary structure presented in chapter VI. The consequence of this dihedral change is pictured in figure VII-1, where the removal of the kink in the B-helix of the crystal structure not only puts this helix in an orientation similar to the model, but also reorients the C-helix, giving a helical arrangement which is consistent with the model and other  $\text{Ca}^{2+}$ -binding protein structures solved in the  $\text{Ca}^{2+}$  form.

**Figure VII-1.** (page 116) Schematic representation of the  $\text{Ca}^{2+}$ -induced straightening of helix-B, and its possible impact on the tertiary fold of NTnC. (I) the  $\text{Ca}^{2+}$ -free crystal structure (Herzberg & James, 1988); (II) the predicted  $\text{Ca}^{2+}$ -saturated structure (Herzberg et al., 1986); (III) same as (I) with helix-B straightened by changing the  $\phi/\psi$  backbone angle of E41 from  $-96/-7$  to  $-66/-41$ . As can be seen by comparing (II) and (III) the straightening of helix-B, which is the predominant  $\text{Ca}^{2+}$ -induced secondary structure change observed by NMR in NTnC, could account for most of the opening of NTnC upon  $\text{Ca}^{2+}$ -binding.

Crystal structure of TnC;  
N-domain in apo state

Proposed model for  
 $\text{Ca}^{2+}$ -saturated structure

Crystal structure of TnC with  
modified  $\phi/\psi$  for E41  
from -96/-7 to -66/-41



From Herzberg & James, 1983

From Herzberg & James, 1983



An important outcome of the present study is the conclusion that there is virtually no change in  $\alpha$ -helical content in the transition from apo to the  $2\text{Ca}^{2+}$  state of the N-domain of TnC. Although previous estimates of the magnitude of the  $\text{Ca}^{2+}$ -induced far UV CD ellipticity changes attributable to the N-domain transition have been somewhat contradictory [10, 11, 12, 13], a recent study [14] on isolated NTnC has shown clearly a very significant increase ( $\sim 23\%$ ) in the negative values of  $[\theta]_{221\text{nm}}$ . This  $\text{Ca}^{2+}$ -induced ellipticity change must now be attributed to features other than an increase in helical content. Among several possible structural factors affecting the relationship between helical content and ellipticity [15], that attributable to helix-helix interaction seems the most plausible for NTnC. As cited in this review, both experimental and theoretical studies suggest a decrease in the CD intensity of  $\sim 10\%$  for two antiparallel helices separated by 7-10 Å compared to a single  $\alpha$ -helix. In the  $\text{Ca}^{2+}$ -induced opening of the structure NTnC [4, chapter VI], the B and C helices move as a unit relative to the N, A and D helices. As a result, the orientations of helix-B and helix-C to those of A and D change from nearly antiparallel to roughly perpendicular. In other words the helix packing in the apo form of NTnC would produce a smaller ellipticity signal than expected assuming only contributions from the secondary structure. Other contributions could include the removal of the kink at residue 41 of helix-B [16],  $\text{Ca}^{2+}$ -induced aggregation and the effects of alterations in the environment of clustered Phe side-chains [15]. However, these are likely to be of lesser significance than contributions arising from reorientation of helices. In any case the present study shows that it is the  $\text{Ca}^{2+}$ -induced tertiary structural changes, not the secondary structural ones, that are responsible for the CD ellipticity change in the N-domain of troponin-C.



---

References

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## CONCLUDING REMARKS

I believe the results presented in this thesis bring the understanding of muscle contraction at the molecular level one step forward. The characterization of the secondary structure brings clarification into the understanding of the CD changes observed in previous studies. This should not be regarded as an attempt to diminish the CD studies, but as data that could be used to enhance the understanding of special cases like this one in CD spectroscopy.

Although the solution structure of the  $\text{Ca}^{2+}$ -saturated regulatory domain of TnC still needs to mature, it clearly demonstrates for the first time that the Herzberg-Moult-James model is a good model. The assignments and the preliminary structure presented here are setting up the stage for the refinement of the NMR structure which will deliver the details of the  $\text{Ca}^{2+}$ -induced structural changes in the regulatory domain of TnC.

Although the complete characterization of the  $\text{Ca}^{2+}$ -induced structural changes in TnC is fascinating, probably more exciting is the fact that the results obtained during my graduate studies provide the essential tools for the structural characterization of NTnC complex with fragments of TnI, a study which will bring understanding in the next step of muscle contraction.

APPENDIX **A****PULSE SEQUENCES CODE****1. HNCA**

```

/* hnca_ct_sl.c - HNCA 3D experiment by Kay et al.; uses a
frequency-shifted pulse on the CO spins; has
RF duality. This experiment does constant time
in the N15 dimension and optional spinlock purge
pulses for suppression of H2O.

Parameters:

sspul = 'y': selects for Trim(x)-Trim(y) sequence at the start
of the pulse sequence
f1180 = 'n': standard t1 timing
        'y': modified t1 timing for t1(1) = half the dwell time
f2180 = 'n': standard t2 timing
        'y': modified t2 timing for t2(1) = half the dwell time
        'n': normal experiment
fad1 = 'y': TPPI axial-peak displacement along t1
        'n': standard phasecycle
fad2 = 'y': TPPI axial-peak displacement along t2 (3D experiment)
        'n': standard phasecycle
satmode = 'y': H1 presaturation during relaxation delay
satfrq = frequency of 1H presaturation for all periods
satdly = saturation time during the relaxation period
satpwr = saturation power for all periods of presaturation with xmtr
hs = 'yn': homospoil pulse (hst) during the d1 relaxation delay
spinlock = flag to check if spin lock purge pulses are to be used
sltime1 = spin lock time in ms of first spin lock period
sltime2 = spin lock time in ms of second spin lock period
          (should be different than sltime1).
slpwr = spin lock power level.
tpwr = power level for 1H transmitter pulses
pw = 90 degree xmtr pulse length for protons (the observed nucleus)
dof = should be set to Ca frequency
pwcalvl = power level for Ca decoupler pulses
pwca = 90 degree decoupler pulse length for Ca at 'pwcalvl'
pwcolvl = power level for C0 decoupler pulses
pwco = 90 degree decoupler pulse length for C0 at 'pwcolvl'
col80 = C=O shaped pulse
hard = hard shaped pulse
pwn15lvl = power level for N15 decoupler pulses
pwn15 = 90 degree decoupler pulse length for N15
dpwr2 = power level for N15 broadband decoupling
inept = inept delay in sequence (NH scalar coupling)
const = constant-time delay
dm = 'nnnnn': no broadband decoupling of N15 during acquisition
     'nnnny': broadband heteronuclear decoupling of N15
           during acquisition
dm2 = 'n': no broadband decoupling of C13 during acquisition

phase = 1,2: hypercomplex experiment with F1 quadrature (complex F1-FT)
phase2 = 1,2: hypercomplex experiment with F2 quadrature (complex F2-FT)

modified from hncoca_ct.c(S.Gagne) 07/04/92

modified to include constant-time in F2 (S.Gagne) 07/04/92
ref.: Grzesiez and Bax, J. Mag. Reson., 96, 432-440 (1992).

modified to include spin lock purge pulses (C. Slupsky), Feb, 1993
*/

```

```

#include <standard.h>
#include <math.h>

#define MIN_DELAY 0.2e-6 /* shortest executable delay */

static int    phs1[2] = {0,2},
              phs2[2] = {1,3},
              phs3[1] = {0},
              phs4[16] = {0,0,0,0,1,1,1,1,2,2,2,2,3,3,3,3},
              phs5[32] = {0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
                          2,2,2,2,2,2,2,2,2,2,2,2,2,2,2,2},
              phs6[32] = {1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,1,
                          3,3,3,3,3,3,3,3,3,3,3,3,3,3,3,3},
              phs7[4] = {0,0,2,2},
              phs8[1] = {1},
              rec[32] = {0,2,2,0,2,0,0,2,0,2,2,0,2,0,0,2,
                          2,0,0,2,0,2,2,0,2,0,0,2,0,2,2,0};

static double d2_init = 0.0,
              d3_init = 0.0;

pulsesequence()
{
    /* VARIABLE DECLARATION */
    char satmode[MAXSTR],
          col80[MAXSTR],
          hard[MAXSTR],
          sspul[MAXSTR],
          spinlck[MAXSTR],
          fad1[MAXSTR],
          fad2[MAXSTR],
          f1180[MAXSTR],
          f2180[MAXSTR];

    int phase,
          phase2,
          satmove,
          t1_counter,
          t2_counter,
          c13dev = DODEV,
          n15dev = DO2DEV;

    double ss,
            t1evol,
            t2evol_1,
            t2evol_2,
            sw1,
            sw2,
            ni2,
            pwcalvl,
            pwca,
            pwcolvl,
            pwco,
            pwn15lvl,
            pwn15,
            satfrq,
            satdly,
            satpwr,
            sltime1,
            sltime2,
            slpwr,
            inept,
            const;

    /* Load variables */
    satfrq = getval("satfrq");
    satdly = getval("satdly");
    satpwr = getval("satpwr");
    sltime1 = getval("sltime1");
    sltime2 = getval("sltime2");
    slpwr = getval("slpwr");
    pwcalvl = getval("pwcalvl");
    pwca = getval("pwca");
    pwcolvl = getval("pwcolvl");
    pwco = getval("pwco");
    pwn15lvl = getval("pwn15lvl");

```

```

pwn15 = getval('pwn15');
inept = getval('inept');
const = getval('const');
ss = getval('ss');
sw1 = getval('sw1');
sw2 = getval('sw2');
ni2 = getval('ni2');
phase = (int) (getval('phase') + 0.5);
phase2 = (int) (getval('phase2') + 0.5);

getstr('sspul', sspul);
getstr('col180', col180);
getstr('hard', hard);
getstr('fad1', fad1);
getstr('fad2', fad2);
getstr('satmode', satmode);
getstr('f1180', f1180);
getstr('f2180', f2180);
getstr('spinlck', spinlck);

/* Load phase cycles */
settable(t1, 2, phs1);
settable(t2, 2, phs2);
settable(t3, 1, phs3);
settable(t4, 16, phs4);
settable(t5, 32, phs5);
settable(t6, 32, phs6);
settable(t7, 4, phs7);
settable(t8, 1, phs8);
settable(t9, 32, rec);

/* Check conditions */
satmove = ( fabs(tof - satfrq) >= 0.1 );

if ( (dm2[A] == 'y') || (dm2[B] == 'y') || (dm2[C] == 'y')
    || (dm2[D] == 'y') )
{
    text_error("'dm2' must be to 'nnnnn' or 'nnnny' for N15 decoupling\n");
    abort(1);
}

if ( (dm[A] == 'y') || (dm[B] == 'y') || (dm[C] == 'y')
    || (dm[D] == 'y') || (dm[E] == 'y') )
{
    text_error("'dm' must be to 'n' for C13\n");
    abort(1);
}

if ( (const) <= (((ni2/sw2)/2)+0.0002) )
{
    text_error("'const' must be larger then '(ni2/sw2)/2'\n");
    abort(1);
}

/* Determine steady-state mode */
if (ss < 0)
{
    ss *= (-1);
    initval(ss, ssval);
    initval(ss, ssctr);
}

/* Add in States-Haberkorn element */
if (phase2 == 2) /* N15 t1 element */
    tsadd(t3, 1, 4);

if (phase == 2) /* C13 t2 element */
    tsadd(t7, 1, 4);

/* Add in FAD */
if (fad2[A] == 'y') /* for N15 */
{
    if (ix == 1)
        d3_init = d3;
}

```

```

    t2_counter = (int) ( (d3 - d3_init)*sw2 + 0.5 );
    if (t2_counter % 2)
    {
        tsadd(t3, 2, 4); /* first N15 90-degree pulse */
        tsadd(t9, 2, 4); /* receiver phase cycle */
    }
}

if (fad1[A] == 'y') /* for C13 */
{
    if (ix == 1)
        d2_init = d2;

    t1_counter = (int) ( (d2 - d2_init)*sw1 + 0.5 );
    if (t1_counter % 2)
    {
        tsadd(t7, 2, 4); /* first C13a 90-degree pulse */
        tsadd(t9, 2, 4); /* receiver phase cycle */
    }
}

/* BEGIN ACTUAL PULSE SEQUENCE CODE */
status(A);
rlpower(tpwr, TODEV); /* H1 hard-pulse power level */
rlpower(pwn15lvl, n15dev); /* N15 hard-pulse power level */
rlpower(pwcolvl, c13dev); /* CO 180 power */

if (sspul[A] == 'y')
{
    rgpulse(200*pw, zero, rof1, 0.0);
    rgpulse(200*pw, one, 0.0, rof2);
}
hsdelay(d1);

/* selective saturation period */
if (satmode[A] == 'y')
{
    if (satmove)
        offset(satfrq, TODEV);

    rlpower(satpwr, TODEV);
    rgpulse(satdly, zero, 4.0e-5, 0.2e-6);
    if (satmove)
        offset(tof, TODEV);

    rlpower(tpwr, TODEV);
    delay(1.0e-5);
}

/* Pulse train */
status(B);
rcvroff();

txphase(zero); /* 1H phase */
dec2phase(t1); /* N15 phase */
decphase(zero); /* C13 phase */
rgpulse(pw, zero, 0.0, 0.0);
delay(inept);

sim3pulse(2*pw, 0.0, 2*pwn15, zero, zero, t1, 0.0, 0.0);
dec2phase(t3); /* N15 phase */
if (spinlck[0] == 'y')
{
    rlpower(slpwr, TODEV);
    delay(inept-POWER_DELAY);
    xmtron();
    delay(sltimel);
    xmtroff();
    rlpower(tpwr, TODEV);
}
else
{
    delay(inept);
}
txphase(t2); /* H1 phase */

```

```

sim3pulse(pw, 0.0, pwn15, t2, zero, t3, 0.0, 0.0);
txphase(zero);
dec2phase(t4);

t1evol = d3; /* N15 is governed by ni2 and sw2 */
if (f2180[0] == 'y')
    t1evol += 0.5/sw2;

if (t1evol < MIN_DELAY)
    t1evol = 0.0;

delay(t1evol/2);
decshaped_pulse(col180, 2*pwco, zero, 0.0, 0.0);

status(C);
decphase(t5);
rlpower(pwcalv1, c13dev);
delay(const - POWER_DELAY);
sim3pulse(2*pw, 2*pwca, 2*pwn15, zero, t5, t4, 0.0, 0.0);
decphase(t7);
dec2phase(t6);
delay(const + 2*pwco - (t1evol/2));
sim3pulse(0.0, pwca, pwn15, zero, t7, t6, 0.0, 0.0);

rlpower(pwcolv1, c13dev);
decphase(zero);
dec2phase(zero);

t2evol_1 = d2/2; /* C13 is governed by ni and sw1 */
t2evol_2 = d2/2; /* C13 is governed by ni and sw1 */
if (f1180[0] == 'y')
{
    t2evol_1 += 0.25/sw1;
    t2evol_2 += 0.25/sw1;
}
if ((t2evol_1 > MIN_DELAY) || (t2evol_2 > MIN_DELAY))
{
    t2evol_1 -= pwca/2 + pwco + POWER_DELAY + WFG_START_DELAY;
    t2evol_2 -= pwca/2 + pwco + POWER_DELAY + WFG_STOP_DELAY;

    if (t2evol_1 < MIN_DELAY)
        t2evol_1 = 0.0;
    if (t2evol_2 < MIN_DELAY)
        t2evol_2 = 0.0;
}

delay(t2evol_1);

sim3shaped_pulse(hard, col180, hard, 2*pw, 2*pwco, 2*pwn15, zero, zero, zero,
0.0, 0.0);

rlpower(pwcalv1, c13dev);
delay(t2evol_2);

sim3pulse(0.0, pwca, pwn15, zero, zero, zero, 0.0, 0.0);
delay(const);
sim3pulse(0.0, 2*pwca, 2*pwn15, zero, zero, zero, 0.0, 0.0);
dec2phase(t8);
delay(const);

status(D);

sim3pulse(pw, 0.0, pwn15, zero, zero, t8, 0.0, 0.0);
dec2phase(zero);
delay(inept);
sim3pulse(2*pw, 0.0, 2*pwn15, zero, zero, zero, 0.0, 0.0);
if (spinlck[0] == 'y')
{
    rlpower(slpwr, TODEV);
    delay(inept-POWER_DELAY);
    xmtron();
    delay(slttime2);
    xmtroff();
    rlpower(tpwr, TODEV);
}

```



---

```
    else
    {
        delay(inept);
    }

    rcvtron();

    rlpower(dpwr2, DO2DEV);

    status(E);
    setreceiver(t9);
}
```

## 2. HNCOCA.

```

/* hncoca_ct.c - HN(CO)CA 3D experiment by Kay et al.; uses a
   frequency-shifted pulse on the Ca spins; has
   RF duality

   no proton decoupling is used(as opposed to hncoca.c)
   constant-time experiment in f2

Parameters:

    sspul = 'y': selects for Trim(x)-Trim(y) sequence at the start
               of the pulse sequence
    f1180 = 'n': standard t1 timing
    f2180 = 'y': modified t1 timing for t1(1) = half the dwell time
    f2180 = 'n': standard t2 timing
    f2180 = 'y': modified t2 timing for t2(1) = half the dwell time
    fad1 = 'n': normal experiment
    fad1 = 'y': TPPI axial-peak displacement along t1
    fad2 = 'n': standard phasecycle
    fad2 = 'y': TPPI axial-peak displacement along t2 (3D experiment)
    satmode = 'y': H1 presaturation during relaxation delay
    satfrq = frequency of 1H presaturation for all periods
    satdly = saturation time during the relaxation period
    satpwr = saturation power for all periods of presaturation with xmtr
    hs = 'yn': homospoil pulse (hst) during the d1 relaxation delay
    tpwr = power level for 1H transmitter pulses
    pw = 90 degree xmtr pulse length for protons (the observed nucleus)
    dof = should be set to C0 frequency
    pwcalvl = power level for Ca decoupler pulses
    pwca = 90 degree decoupler pulse length for Ca at 'pwcalvl'
    cashape = frequency-shifted pulse for Ca excitation
    pwca2lvl = power level for Ca 180 decoupler pulses
    pwca2 = 180 degree decoupler pulse length for Ca at 'pwcalvl'
    cashape2 = frequency-shifted pulse for Ca 180 excitation
    pwcolvl = power level for C0 decoupler pulses
    pwco = 90 degree decoupler pulse length for C0 at 'pwcolvl'
    pwco2lvl = power level for shorter C0 pulse
    pwco2 = 90 degree decoupler pulse length for C0 at 'pwco2lvl'
    pwn15lvl = power level for N15 decoupler pulses
    pwn15 = 90 degree decoupler pulse length for N15
    dpwr2 = power level for N15 broadband decoupling
    inept = inept delay in sequence (NH scalar coupling; ~2.4 ms)
    tau1 = first delay in sequence (~8.3 ms)
    tau2 = second delay in sequence (~3.0 ms)
    tau3 = third delay in sequence (~5.5 ms)
    dm2 = 'nnnnn': no broadband decoupling of N15 during acquisition
    dm2 = 'nnnny': broadband heteronuclear decoupling of N15
               during acquisition
    dm = 'n': no broadband decoupling of C13 during acquisition

    phase = 1,2: hypercomplex experiment with F1 quadrature (complex F1-FT)
    phase2 = 1,2: hypercomplex experiment with F2 quadrature (complex F2-FT)

modified from hncocalFSP.c(S.Farmer,Varian) 1/27/92

modified from hncocaxx.c to include constant-time in F2 (S.Gagne) 07/01/92
ref.: Grzesiez and Bax, J. Magn. Reson., 96, 432-440 (1992).

NOTE :
-----

SHAPED PULSE : carrier on C=O and shaped pulse on Ca

*/

#include <standard.h>
#include <math.h>

#define MIN_DELAY 0.2e-6 /* shortest executable delay */

static int phs1[4] = {0,0,2,2},

```

```

        phs2[2] = {0,2},
        phs3[8] = {0,0,0,0,2,2,2,2},
        phs5[16] = {0,0,0,0,0,0,0,0,2,2,2,2,2,2,2,2},
        rec[16] = {0,2,2,0,2,0,0,2,2,0,0,2,0,2,2,0};

static double d2_init = 0.0,
              d3_init = 0.0;

pulsessequence()
{
/* VARIABLE DECLARATION */
char          satmode[MAXSTR],
              sspul[MAXSTR],
              fad1[MAXSTR],
              fad2[MAXSTR],
              f1180[MAXSTR],
              f2180[MAXSTR],
              cashape[MAXSTR],
              cashape2[MAXSTR];

int           phase,
              phase2,
              satmove,
              t1_counter,
              t2_counter,
              cl3dev = DODEV,
              nl5dev = DO2DEV;

double        ss,
              t1evol,
              t2evol_1,
              t2evol_2,
              sw1,
              sw2,
              ni2,

              pwcalvl,
              pwca,
              pwca2lvl,
              pwca2,
              pwcolvl,
              pwco,
              pwco2lvl,
              pwco2,
              pwn15lvl,
              pwn15,
              satfrq,
              satdly,
              satpwr,

              inept,
              tau2,
              tau1,
              tau3;

/* Load variables */
satfrq = getval("satfrq");
satdly = getval("satdly");
satpwr = getval("satpwr");
pwcalvl = getval("pwcalvl");
pwca = getval("pwca");
pwca2lvl = getval("pwca2lvl");
pwca2 = getval("pwca2");
pwcolvl = getval("pwcolvl");
pwco = getval("pwco");
pwco2lvl = getval("pwco2lvl");
pwco2 = getval("pwco2");
pwn15lvl = getval("pwn15lvl");
pwn15 = getval("pwn15");
inept = getval("inept");
tau2 = getval("tau2");
tau1 = getval("tau1");
tau3 = getval("tau3");
ss = getval("ss");
sw1 = getval("sw1");
sw2 = getval("sw2");
ni2 = getval("ni2");
phase = (int) (getval("phase") + 0.5);
phase2 = (int) (getval("phase2") + 0.5);

```

```

    getstr('cashape', cashape);
    getstr('cashape2', cashape2);
    getstr('sspul', sspul);
    getstr('fad1', fad1);
    getstr('fad2', fad2);
    getstr('satmode', satmode);
    getstr('f1180', f1180);
    getstr('f2180', f2180);

/* Load phase cycles */
settable(t1, 4, phs1);
settable(t2, 2, phs2);
settable(t3, 8, phs3);
settable(t4, 16, rec);
settable(t5, 16, phs5);

/* Check conditions */
satmove = ( fabs(tof - satfrq) >= 0.1 );

if ( (dm2[A] == 'y') || (dm2[B] == 'y') || (dm2[C] == 'y')
    || (dm2[D] == 'y') )
{
    text_error('dm2' must be to 'nnnnn' or 'nnnny' for N15 decoupling\n');
    abort(1);
}

if ( (dm[A] == 'y') || (dm[B] == 'y') || (dm[C] == 'y')
    || (dm[D] == 'y') || (dm[E] == 'y') )
{
    text_error('dm' must be to 'n' for C13\n');
    abort(1);
}

if ( (tau2+tau1) <= ((ni2/sw2)/2) )
{
    text_error('tau2+tau1' must be larger then '(ni2/sw2)/2');
    abort(1);
}

/* Determine steady-state mode */
if (ss < 0)
{
    ss *= (-1);
    initval(ss, ssva1);
    initval(ss, ssctr);
}

/* Add in States-Haberkorn element */
if (phase2 == 2) /* N15 t1 element */
    tsadd(t2, 1, 4);

if (phase == 2) /* C13 t2 element */
    tsadd(t3, 1, 4);

/* Add in FAD */
if (fad2[A] == 'y') /* for N15 */
{
    if (ix == 1)
        d3_init = d3;

    t2_counter = (int) ( (d3 - d3_init)*sw2 + 0.5 );
    if (t2_counter % 2)
    {
        tsadd(t2, 2, 4); /* first N15 90-degree pulse */
        tsadd(t4, 2, 4); /* receiver phase cycle */
    }
}

if (fad1[A] == 'y') /* for C13 */
{
    if (ix == 1)
        d2_init = d2;

    t1_counter = (int) ( (d2 - d2_init)*sw1 + 0.5 );
}

```

```

        if (t1_counter % 2)
        {
            tsadd(t3, 2, 4);          /* first C13a 90-degree pulse */
            tsadd(t4, 2, 4);          /* receiver phase cycle */
        }
    }

/* BEGIN ACTUAL PULSE SEQUENCE CODE */
status(A);
rlpower(tpwr, TODEV);              /* H1 hard-pulse power level */
rlpower(pwn15lvl, n15dev);          /* N15 hard-pulse power level */
rlpower(pwca2lvl, c13dev);          /* Ca 180 power */

if (sspul[A] == 'y')
{
    rgpulse(200*pw, zero, rof1, 0.0);
    rgpulse(200*pw, one, 0.0, rof2);
}
hsdelay(d1);

/* selective saturation period */
if (satmode[A] == 'y')
{
    if (satmove)
        offset(satfrq, TODEV);

    rlpower(satpwr, TODEV);
    rgpulse(satdly, zero, 4.0e-5, 0.2e-6);
    if (satmove)
        offset(tof, TODEV);

    rlpower(tpwr, TODEV);
    delay(1.0e-5);
}

/* Pulse train */
status(B);
rcvroff();

rgpulse(pw, zero, rof1, 0.0);
dec2phase(t1);                    /* N15 phase */
decphase(zero);                   /* C13 phase */
delay(inept);

sim3pulse(2*pw, 0.0, 2*pwn15, zero, zero, t1, 0.0, 0.0);
txphase(t1);                      /* H1 phase */
dec2phase(t2);                   /* N15 phase */
delay(inept);

getelem(t1, ct, v14);
add(one, v14, v14);

sim3pulse(pw, 0.0, pwn15, v14, zero, t2, 0.0, 0.0);
txphase(zero);
dec2phase(zero);

tlevol = d3; /* N15 is governed by n12 and sw2 */
if (f2180[0] == 'y')
    tlevol += 0.5/sw2;

if (tlevol > MIN_DELAY)
{
    tlevol -= pwn15 + pwca2 + 2*WFG_START_DELAY;
    if (tlevol < MIN_DELAY)
        tlevol = 0.0;
}

delay(tlevol/2);
decshaped_pulse(cashape2, pwca2, zero, 0.0, 0.0);
delay(tau1 - pwca2/2 - WFG_STOP_DELAY - pw);
rgpulse(2*pw, zero, 0.0, 0.0);

status(C);
delay(tau2/2 - pw);
rlpower(pwcolvl, c13dev);

```

```

delay( (tau2/2) - POWER_DELAY - pwco );
sim3pulse(0.0, 2*pwco, 2*pwn15, zero, zero, zero, 0.0, 0.0);
delay(tau2 + tau1 - pwco - pwco/2 - (tlevol/2) - pwn15/2 - pwca/2 -
      WFG_START_DELAY);
sim3pulse(0.0, pwco, pwn15, zero, zero, zero, 0.0, 0.0);

delay(tau3/2 - pwco/2);
rlpower(pwcalv1, c13dev);
decphase(t3);
delay( (tau3/2) - POWER_DELAY - WFG_START_DELAY - pwca/2);
decshaped_pulse(cashape, pwca, t3, 0.0, 0.0);

t2evol_1 = t2evol_2 = d2/2; /* C13 is governed by ni and sw */
if (fil80[0] == 'y')
{
    t2evol_1 += 0.25/sw1;
    t2evol_2 += 0.25/sw1;
}

if ( (t2evol_1 > MIN_DELAY) || (t2evol_2 > MIN_DELAY) )
{
    t2evol_1 -= pwca/2 + pwco2 + POWER_DELAY + WFG_STOP_DELAY;
    t2evol_2 -= pwca/2 + pwco2 + POWER_DELAY + WFG_START_DELAY;

    if (t2evol_1 < MIN_DELAY)
        t2evol_1 = 0.0;
    if (t2evol_2 < MIN_DELAY)
        t2evol_2 = 0.0;
}

decphase(zero);
rlpower(pwco2lv1, c13dev);
delay(t2evol_1);

simpulse(2*pw, 2*pwco2, zero, zero, 0.0, 0.0);

rlpower(pwcalv1, c13dev);
delay(t2evol_2);

decshaped_pulse(cashape, pwca, zero, 0.0, 0.0);
delay( (tau3/2) - WFG_STOP_DELAY - pwca/2 );
rlpower(pwcolv1, c13dev);
delay( (tau3/2) - POWER_DELAY - pwco/2 );

status(D);
sim3pulse(0.0, pwco, pwn15, zero, zero, t5, 0.0, 0.0);
delay(tau1 + tau2 - pwco/2 - pwco);
sim3pulse(0.0, 2*pwco, 2*pwn15, zero, zero, zero, 0.0, 0.0);
delay(tau1 - pwco - pw);

rgpulse(2*pw, zero, 0.0, 0.0);
delay(tau2 - pw - pwn15/2);
sim3pulse(pw, 0.0, pwn15, zero, zero, zero, 0.0, 0.0);
delay(inept);
sim3pulse(2*pw, 0.0, 2*pwn15, zero, zero, zero, 0.0, 0.0);

rlpower( ((n15dev == DODEV) ? dpwr : dpwr2), n15dev);
rlpower( ((c13dev == DODEV) ? dpwr : dpwr2), c13dev);
delay(rof2);
rcvtron();
delay(inept - rof2 - 2*POWER_DELAY);

status(E);
setreceiver(t4);
}

```

## 3. HNCO.

```

/* hnco_ct.c      - HNCO 3D experiment with constant-time evolution in F1 (15N)
                  - 13C carrier (dec #1) on C=O and shifted pulse on Ca
                  - ref.: Grzesiez and Bax, J. Magn. Reson., 96, 432-440 (1992).
                  - programmed by S.M. Gagne (6 July 1992)
                  - last revised (13 August 1992)

```

## Parameters:

```

sspul = 'y': selects for Trim(x)-Trim(y) sequence at the start
           of the pulse sequence
f1180 = 'n': standard t1 timing
        'y': modified t1 timing for t1(1) = half the dwell time
f2180 = 'n': standard t2 timing
        'y': modified t2 timing for t2(1) = half the dwell time
        'n': normal experiment
fad1 = 'y': TPPI axial-peak displacement along t1
        'n': standard phasecycle
fad2 = 'y': TPPI axial-peak displacement along t2 (3D experiment)
        'n': standard phasecycle
satmode = 'y': H1 presaturation during relaxation delay
satfrq = frequency of 1H presaturation for all periods
satdly = saturation time during the relaxation period
satpwr = saturation power for all periods of presaturation with xmtr
hs = 'yn': homospoil pulse (hst) during the d1 relaxation delay
tpwr = power level for 1H transmitter pulses
pw = 90 degree xmtr pulse length for protons (the observed nucleus)
hard = hard shape-pulse for 1H and 15N
dof = should be set to C0 frequency
pwcalv1 = power level for Ca decoupler pulses
pwcal80 = 180 degree decoupler pulse length for Ca at 'pwcalv1'
cashape = frequency-shifted pulse for 180 degree Ca excitation
          (see cashape note below)
pwcolv1 = power level for C0 decoupler pulses
pwco = 90 degree decoupler pulse length for C0 at 'pwcolv1'
pwn15l1 = power level for N15 decoupler pulses
pwn15 = 90 degree decoupler pulse length for N15
dpwr2 = power level for N15 broadband decoupling
inept = inept delay in sequence (1/4*NH scalar coupling) (about 2.45ms)
tau2 = second delay in sequence ( about 3ms )
tau1 = first delay in sequence ( about 10ms )
dm2 = 'nnnn': no broadband decoupling of N15 during acquisition
      'nnnny': broadband heteronuclear decoupling of N15
           during acquisition
dm = 'n': no broadband decoupling of C13 during acquisition
nt = multiple of 16

phase = 1,2: hypercomplex experiment with F1 quadrature (complex F1-FT)
phase2 = 1,2: hypercomplex experiment with F2 quadrature (complex F2-FT)

```

## NOTES:

```

cashape : the first null of the 180 Ca shifted pulse should correspond to
           the frequency of the C=O carrier (dof)

constant time : the constant-time evolution period is equal to 2*(tau1+tau2)
                and should be set to an odd multiple of 1/(2JNH) (~26 ms)

Sensitivity : this experiment is more sensitive then the HNCA or
              HN(CO)CA ; less transients are necessary for the HNCO.

Suggestions from SMG :
    inept, tau1, tau2 optimization : Start with the default values then
    optimize on the first increment (ni=ni2=phase=phase2=1) in
    order to maximize the intensity of the signal. Optimize first
    inept, then tau2 and finally tau1.

```

```

*/

```



```

#include <standard.h>
#include <math.h>

#define MIN_DELAY 0.2e-6 /* shortest executable delay */
#define SIM3SH_START_DELAY 30.2e-6
#define SIM3SH_STOP_DELAY 12.2e-6

static int    phs1[2] = {0,2},
              phs2[2] = {1,3},
              phs3[1] = {0},
              phs4[16] = {0,0,0,0,1,1,1,1,2,2,2,2,3,3,3,3},
              phs5[4] = {0,0,2,2},
              phs6[2] = {0,2},
              rec[8] = {0,0,2,2,2,2,0,0};

static double d2_init = 0.0,
              d3_init = 0.0;

pulsesequance()
{
/* VARIABLE DECLARATION */
  char      satmode[MAXSTR],
            sspul[MAXSTR],
            fad1[MAXSTR],
            fad2[MAXSTR],
            f1180[MAXSTR],
            f2180[MAXSTR],
            hard[MAXSTR],
            cashape[MAXSTR];

  int       phase,
            phase2,
            satmove,
            t1_counter,
            t2_counter,
            c13dev = DODEV,
            n15dev = DO2DEV;

  double    ss,
            t1evol,
            t2evol_1,
            t2evol_2,
            sw1,
            sw2,
            ni2,

            pwcalvl,
            pwcal80,
            pwcolvl,
            pwco,
            pwn15lvl,
            pwn15,
            satfrq,
            satdly,
            satpwr,

            inept,
            tau2,
            tau1;

/* Load variables */
  satfrq = getval("satfrq");
  satdly = getval("satdly");
  satpwr = getval("satpwr");
  pwcalvl = getval("pwcalvl");
  pwcal80 = getval("pwcal80");
  pwcolvl = getval("pwcolvl");
  pwco = getval("pwco");
  pwn15lvl = getval("pwn15lvl");
  pwn15 = getval("pwn15");
  inept = getval("inept");
  tau2 = getval("tau2");
  tau1 = getval("tau1");
  ss = getval("ss");
  sw1 = getval("sw1");
  sw2 = getval("sw2");
  ni2 = getval("ni2");
  phase = (int) (getval("phase") + 0.5);
  phase2 = (int) (getval("phase2") + 0.5);

```

```

getstr('cshape', cshape);
getstr('hard', hard);
getstr('sspul', sspul);
getstr('fad1', fad1);
getstr('fad2', fad2);
getstr('satmode', satmode);
getstr('f1180', f1180);
getstr('f2180', f2180);

/* Load phase cycles */
settable(t1, 2, phs1);
settable(t2, 2, phs2);
settable(t3, 1, phs3);
settable(t4, 16, phs4);
settable(t5, 4, phs5);
settable(t6, 2, phs6);
settable(t7, 8, rec);

/* Check conditions */
satmove = ( fabs(tof - satfrq) >= 0.1 );

if ( (dm2[A] == 'y') || (dm2[B] == 'y') || (dm2[C] == 'y')
    || (dm2[D] == 'y') )
{
    text_error('dm2 must be to 'nnnnn' or 'nnnny' for N15 decoupling\n');
    abort(1);
}

if ( (dm[A] == 'y') || (dm[B] == 'y') || (dm[C] == 'y')
    || (dm[D] == 'y') || (dm[E] == 'y') )
{
    text_error('dm must be to 'n' for C13\n');
    abort(1);
}

if ( (tau2+tau1) <= ((ni2/sw2)/2) )
{
    text_error('tau2+tau1 must be larger then '(ni2/sw2)/2''');
    abort(1);
}

/* Determine steady-state mode */
if (ss < 0)
{
    ss *= (-1);
    initval(ss, ssval);
    initval(ss, ssctr);
}

/* Add in States-Haberkorn element */
if (phase2 == 2) /* N15 t1 element */
    tsadd(t3, 1, 4);

if (phase == 2) /* C13 t2 element */
    tsadd(t5, 1, 4);

/* Add in FAD */
if (fad2[A] == 'y') /* for N15 */
{
    if (ix == 1)
        d3_init = d3;

    t2_counter = (int) ( (d3 - d3_init)*sw2 + 0.5 );
    if (t2_counter % 2)
    {
        tsadd(t3, 2, 4); /* first N15 90-degree pulse */
        tsadd(t7, 2, 4); /* receiver phase cycle */
    }
}

if (fad1[A] == 'y') /* for C13 */
{
    if (ix == 1)

```

```

        d2_init = d2;

        tl_counter = (int) ( (d2 - d2_init)*sw1 + 0.5 );
        if (tl_counter % 2)
        {
            tsadd(t5, 2, 4);          /* first C13a 90-degree pulse */
            tsadd(t7, 2, 4);          /* receiver phase cycle */
        }
    }

/* BEGIN ACTUAL PULSE SEQUENCE CODE */
status(A);
    rlpower(tpwr, TODEV);          /* H1 hard-pulse power level */
    rlpower(pwn15lv1, n15dev);      /* N15 hard-pulse power level */
    rlpower(pwcalv1, c13dev);       /* Ca 180 power */

    if (sspul[A] == 'y')
    {
        rgpulse(200*pw, zero, rof1, 0.0);
        rgpulse(200*pw, one, 0.0, rof2);
    }
    hsdelay(d1);

/* selective saturation period */
    if (satmode[A] == 'y')
    {
        if (satmove)
            offset(satfrq, TODEV);

        rlpower(satpwr, TODEV);
        rgpulse(satdly, zero, 4.0e-5, 0.2e-6);
        if (satmove)
            offset(tof, TODEV);

        rlpower(tpwr, TODEV);
        delay(1.0e-5);
    }

/* Pulse train */
status(B);
    rcvroff();

    rgpulse(pw, zero, rof1, 0.0);
    dec2phase(t1);                  /* N15 phase */
    decphase(zero);                 /* C13 phase */
    delay(inept);

    sim3pulse(2*pw,0.0, 2*pwn15, zero,zero, t1, 0.0, 0.0);
    txphase(t2);                    /* H1 phase */
    dec2phase(t3);                  /* N15 phase */
    delay(inept);

    sim3pulse(pw,0.0, pwn15, t2,zero, t3, 0.0, 0.0);
    txphase(zero);
    dec2phase(t4);

    t1evol = d3; /* N15 is governed by ni2 and sw2 */
    if (f2180[0] == 'y')
        t1evol += 0.5/sw2;

    if (t1evol > MIN_DELAY)
    {
        t1evol -= pwn15 + pwcal80 + 2*WFG_START_DELAY ;
        if (t1evol < MIN_DELAY)
            t1evol = 0.0;
    }

    delay(t1evol/2);
    decshaped_pulse(cashape,pwcal80,zero,0.0,0.0);

status(C);
    delay( (tau1 + tau2)/2 - pwcal80/2 - WFG_STOP_DELAY);
    rlpower(pwcolv1,c13dev);
    delay( (tau1 + tau2)/2 - POWER_DELAY );
    sim3pulse(2*pw, 2*pwco, 2*pwn15, zero, zero, t4,0.0,0.0);

```

```

        decphase(t5);
        dec2phase(t6);
        delay(tau1 + tau2 - (t1evol/2 + pwn15/2 + pwca180/2 + WFG_START_DELAY -
        pwco/2));
        sim3pulse(0.0,pwco,pwn15, zero, t5, t6, 0.0, 0.0);

        rlpower(pwcalv1, c13dev);
        decphase(zero);
        dec2phase(zero);

        t2evol_1 = t2evol_2 = d2/2; /* C13 is governed by n1 and sw */
        if (f1180[0] == 'y')
        {
            t2evol_1 += 0.25/sw1;
            t2evol_2 += 0.25/sw1;
        }

        if ( (t2evol_1 > MIN_DELAY) || (t2evol_2 > MIN_DELAY) )
        {
            t2evol_1 -= pwco/2 + pwca180/2 + POWER_DELAY + SIM3SH_START_DELAY;
            t2evol_2 -= pwco/2 + pwca180/2 + POWER_DELAY + SIM3SH_STOP_DELAY;

            if (t2evol_1 < MIN_DELAY)
                t2evol_1 = 0.0;
            if (t2evol_2 < MIN_DELAY)
                t2evol_2 = 0.0;
        }

        delay(t2evol_1);

        sim3shaped_pulse(hard,cashape,hard,2*pw, pwca180, 2*pwn15, zero, zero,
        zero,0.0, 0.0);

        rlpower(pwcolv1, c13dev);
        delay(t2evol_2);

status(D);
        sim3pulse(0.0,pwco,pwn15,zero,zero, zero, 0.0, 0.0);
        delay(tau1 + tau2 - pwco/2);
        sim3pulse(0.0,2*pwco,2*pwn15,zero, zero, zero, 0.0, 0.0);
        delay(tau1 - pw);

        rgpulse(2*pw, zero, 0.0, 0.0);
        delay(tau2 - pw -pwn15/2);
        sim3pulse(pw,0.0, pwn15, zero,zero, zero, 0.0, 0.0);
        delay(inept);
        sim3pulse(2*pw,0.0, 2*pwn15, zero, zero,zero, 0.0, 0.0);

        rlpower( ((n15dev == DODEV) ? dpwr : dpwr2), n15dev);
        rlpower( ((c13dev == DODEV) ? dpwr : dpwr2), c13dev);
        delay(rof2);
        rcvtron();
        delay(inept - rof2 - 2*POWER_DELAY);

status(E);
        setreceiver(t7);
}

```

## 4. HCACO.

```
/* hcaco_ct.c - HCACO 3D experiment with constant-time evolution in F1 (Ca)
- 13C carrier (dec #1) on Ca and shifted pulse on C=O
- ref.: 1. R. Powers, A.M. Gronenborn, G.M. Clore, and A. Bax,
      J. Magn. Reson., 94, 209-213 (1991).
      2. L.E. Kay, M. Ikura, R. Tschudin, and A. Bax,
      J. Magn. Res., 89, 496-514.
- written for spectrometer with only three channel, so used
  consecutive Ca/C=O instead of simultaneous ones.
- programmed by S.M. Gagne (24 August 1992)
- last revised by S.M. Gagne (28 september 1992)
```

## Parameters:

```
sspul = 'y': selects for Trim(x)-Trim(y) sequence at the start
           of the pulse sequence
f1180 = 'n': standard t1 timing
           'y': modified t1 timing for t1(1) = half the dwell time
f2180 = 'n': standard t2 timing
           'y': modified t2 timing for t2(1) = half the dwell time
           'n': normal experiment
fad1 = 'y': TPPI axial-peak displacement along t1
           'n': standard phasecycle
fad2 = 'y': TPPI axial-peak displacement along t2 (3D experiment)
           'n': standard phasecycle
satmode = 'y': H1 presaturation during relaxation delay
satfrq = frequency of 1H presaturation for all periods
satdly = saturation time during the relaxation period (see NOTES below)
satpwr = saturation power for all periods of presaturation with xmtr
        (see NOTES below)
hs = 'yn': homospoil pulse (hst) during the d1 relaxation delay
tpwr = power level for 1H transmitter pulses
pw = 90 degree xmtr pulse length for protons (the observed nucleus)
hard = hard shape-pulse for 1H
dof = should be set to Ca frequency
pwcalvl = power level for Ca decoupler pulses
pwca = 90 degree decoupler pulse length for Ca at 'pwcalvl'
co180 = C=O 180 frequency-shifted pulse (see NOTES below)
co90 = C=O 90 frequency-shifted pulse (see NOTES below)

dpwr2 = power level for N15 broadband decoupling
inept = inept delay in sequence (1/4*NH scalar coupling) (about 2.45ms)
constT = half of the constant-time duration (see NOTES below)
dm2 = 'nnnn': no broadband decoupling of N15
      'nnynn': broadband heteronuclear decoupling of N15
           during t1/t2 evolution period
dm = 'nnnnn': no broadband decoupling of C13 during acquisition
      'nnnny': broadband heteronuclear decoupling of C13
           during acquisition
nt = multiple of 16

phase = 1,2: hypercomplex experiment with F1 quadrature (complex F1-FT)
phase2 = 1,2: hypercomplex experiment with F2 quadrature (complex F2-FT)
```

## NOTES:

co180,co90 : the first null of the C=O shifted pulse should correspond to the frequency of the Ca carrier (dof). If the frequency shift is set to 18181.8 Hz (for a pwco90=55us), a sw2 (C=O) of 1818.18 will have the proper number of folding and usually covers all the backbone C=O.

constant time : the constant-time evolution period is equal to 2\*(constT). Resolution in the t1 dimension is limited by the fact that the t1 acquisition period is restricted to the constant-time duration (2\*constT). As a reasonable compromise between high sensitivity and acceptable resolution, a constant-time duration of 7ms is used (constT=3.5ms). 7ms also correspond to  $\sim 1/(J_{\text{HAc}})$ , so that the Ca spin will be antiphase with respect to the Ha at the end of the time 2\*constT.

water suppression : since the HCACO is done in D2O, no water suppression or very low satpwr can be used with this experiment.

d1/satdly : d1 (or satdly if water suppression is used) can be shorter than usual, because of the relatively short Ha T1 (~0.7s). A relaxation delay of about 0.8s can usually be used without any problem.

Sensitivity : this experiment is a lot more sensitive than the HNCA or HN(CO)CA. Less transients are necessary and since the spectral width in C=O is usually small, less increment in F2 (n1) are necessary.

-----  
\*/

```
#include <standard.h>
#include <math.h>
```

```
#define MIN_DELAY 0.2e-6 /* shortest executable delay */
#define SIMSH_START_DELAY 20.2e-6
#define SIMSH_STOP_DELAY 8.2e-6
```

```
static int
    phs1[8] = {0,0,0,0,2,2,2,2},
    phs2[1] = {0},
    phs3[16] = {0,0,0,0,0,0,0,0,2,2,2,2,2,2,2,2},
    phs4[8] = {1,1,1,1,3,3,3,3},
    phs5[2] = {0,2},
    phs6[4] = {0,0,2,2},
    rec[8] = {0,2,2,0,2,0,0,2};
```

```
static double
    d2_init = 0.0,
    d3_init = 0.0;
```

```
pulsesequance()
{
    /* VARIABLE DECLARATION */
    char
        satmode[MAXSTR],
        sspul[MAXSTR],
        fad1[MAXSTR],
        fad2[MAXSTR],
        f1180[MAXSTR],
        f2180[MAXSTR],
        co180[MAXSTR],
        co90[MAXSTR];

    int
        phase,
        phase2,
        satmove,
        t1_counter,
        t2_counter,
        c13dev = DODEV,
        n15dev = DO2DEV;

    double
        ss,
        t1evol,
        t2evol_1,
        t2evol_2,
        sw1,
        sw2,
        n1,
        pwcalv1,
        pwca,
        satfrq,
        satdly,
        satpwr,
        inept,
        constT;
```

```
/* Load variables */
    satfrq = getval("satfrq");
```

```

satdly = getval('satdly');
satpwr = getval('satpwr');
pwcav1 = getval('pwcav1');
pwca = getval('pwca');
inept = getval('inept');
constT = getval('constT');
ss = getval('ss');
sw1 = getval('sw1');
sw2 = getval('sw2');
ni = getval('ni');
phase = (int) (getval('phase') + 0.5);
phase2 = (int) (getval('phase2') + 0.5);

getstr('col80', col80);
getstr('co90', co90);
getstr('sspul', sspul);
getstr('fad1', fad1);
getstr('fad2', fad2);
getstr('satmode', satmode);
getstr('f1180', f1180);
getstr('f2180', f2180);

/* Load phase cycles */
settable(t1, 8, phs1);
settable(t2, 1, phs2);
settable(t3, 16, phs3);
settable(t4, 8, phs4);
settable(t5, 2, phs5);
settable(t6, 4, phs6);
settable(t7, 8, rec);

/* Check conditions */
satmove = ( fabs(tof - satfrq) >= 0.1 );

if ( (dm2[A] == 'y') || (dm2[B] == 'y') || (dm2[D] == 'y')
    || (dm2[E] == 'y') )
{
    text_error('dm2 must be to 'nnnnn' or 'nnynn' for N15 decoupling\n');
    abort(1);
}

if ( (dm[A] == 'y') || (dm[B] == 'y') || (dm[C] == 'y')
    || (dm[D] == 'y') )
{
    text_error('dm must be to 'nnnnn' or 'nnnny' for C13\n');
    abort(1);
}

if ( (constT) <= (.0001 + (ni/sw1)/2) )
{
    text_error('constT must be larger then '(ni/sw1)/2''');
    abort(1);
}

/* Determine steady-state mode */
if (ss < 0)
{
    ss *= (-1);
    initval(ss, ssva1);
    initval(ss, ssctr);
}

/* Add in States-Haberkorn element */

if (phase == 2)          /* Ca t1 element */
    tsadd(t2, 1, 4);

if (phase2 == 2)         /* C=O t2 element */
    tsadd(t5, 1, 4);

/* Add in FAD */
if (fad1[A] == 'y')      /* for Ca */
{
    if (ix == 1)

```



```

        d2_init = d2;
        t1_counter = (int) ( (d2 - d2_init)*sw1 + 0.5 );
        if (t1_counter % 2)
        {
            tsadd(t2, 2, 4); /* first Ca 90-degree pulse */
            tsadd(t7, 2, 4); /* receiver phase cycle */
        }
    }

    if (fad2[A] == 'y') /* for C=O */
    {
        if (ix == 1)
            d3_init = d3;

        t2_counter = (int) ( (d3 - d3_init)*sw2 + 0.5 );
        if (t2_counter % 2)
        {
            tsadd(t5, 2, 4); /* first C=O 90-degree pulse */
            tsadd(t7, 2, 4); /* receiver phase cycle */
        }
    }

/* BEGIN ACTUAL PULSE SEQUENCE CODE */
status(A);
    rlpower(tpwr, TODEV); /* H1 hard-pulse power level */
    rlpower(dpwr2, n15dev); /* N15 decoupling power level */
    rlpower(pwcalv1, c13dev); /* Ca power level */

    if (sspul[A] == 'y')
    {
        rgpulse(200*pw, zero, rof1, 0.0);
        rgpulse(200*pw, one, 0.0, rof2);
    }
    hsdelay(d1);

/* selective saturation period */
    if (satmode[A] == 'y')
    {
        if (satmove)
            offset(satfrq, TODEV);

        rlpower(satpwr, TODEV);
        rgpulse(satdly, zero, 4.0e-5, 0.2e-6);
        if (satmove)
            offset(tof, TODEV);

        rlpower(tpwr, TODEV);
        delay(1.0e-5);
    }

/* Pulse train */
status(B);
    rcvroff();

    rgpulse(pw, zero, rof1, 0.0);
    decphase(t1); /* Ca phase */
    delay(inept);

    simpulse(2*pw, 2*pwca, zero, t1, 0.0, 0.0);
    txphase(one); /* H1 phase */
    decphase(t2);
    delay(inept);

    simpulse(pw, pwca, one, t2, 0.0, 0.0);
    txphase(zero);
    decphase(t3);

    t1evol = d2; /* Ca is governed by n1 and sw */
    if (f1180[0] == 'y')
        t1evol += 0.5/sw1;

status(C);
    delay(constT - t1evol/2 - WFG_START_DELAY);
    simpulse(2*pw, 2*pwca, zero, t3, 0.0, 0.0);

```

```

decphase(zero);
decshaped_pulse(co180, 2*pwca, zero, 0.0,0.0);
decphase(t4);
delay(constT + t1evol/2 - WFG_STOP_DELAY - WFG_START_DELAY);
decrpulse(pwca, t4, 0.0,0.0);
decphase(t5);
decshaped_pulse(co90, pwca, t5, 0.0,0.0);
decphase(t3);

t2evol_1 = t2evol_2 = d3/2; /* C=0 is governed by n12 and sw2 */
if (f1180[0] == 'y')
{
    t2evol_1 += 0.25/sw2;
    t2evol_2 += 0.25/sw2;
}

if ((t2evol_1 > MIN_DELAY) || (t2evol_2 > MIN_DELAY))
{
    t2evol_1 -= pwca/2 + pwca + WFG_STOP_DELAY;
    t2evol_2 -= pwca/2 + pwca + WFG_START_DELAY;

    if (t2evol_1 < MIN_DELAY)
        t2evol_1 = 0.0;
    if (t2evol_2 < MIN_DELAY)
        t2evol_2 = 0.0;
}

delay(t2evol_1);

simpulse(2*pw,2*pwca, zero,t3, 0.0,0.0);
decphase(t6);
delay(t2evol_2);

status(D);
decshaped_pulse(co90,pwca, t6, 0.0,0.0);
decphase(one);
decrpulse(pwca, one, 0.0,0.0);
delay(constT - WFG_STOP_DELAY - WFG_START_DELAY);
decphase(zero);
decshaped_pulse(co180,2*pwca, zero, 0.0,0.0);
decrpulse(2*pwca, zero, 0.0,0.0);
delay(constT - WFG_STOP_DELAY);
simpulse(pw,pwca, zero,zero, 0.0,0.0);
delay(inept);
simpulse(2*pw,2*pwca, zero,zero, 0.0,0.0);

rlpower( (n15dev == DODEV) ? dpwr : dpwr2), n15dev);
rlpower( (c13dev == DODEV) ? dpwr : dpwr2), c13dev);
delay(rof2);
rcvtron();
delay(inept - rof2 - 2*POWER_DELAY);

status(E);
setreceiver(t7);
}

```

5. <sup>15</sup>N-edited NOESY HMQC.

```

/* nosyhmqc3rf3d - NOESY-HMQC 3D sequence
   - written in hypercomplex phase sensitive mode only
   uses the 2nd decoupler for the X pulses

Sequence:
NOESY-HMQC:

status :      A-|B|----C-----|-----D-----|E--
1H :      90-t1-90-mix-90-1/2J-      180      -1/2J- Acq (t3)
X :      BB      90-t2/2-      -t2/2-90      BB
phtable :      t1      t3      t3      t2      t3      t3      t4 or t5

Parameters:
      d2 = First evolution time
      d3 = Second evolution time
      mix = NOESY mixing time.
      pwx2lvl = power level for X pulses
      pwx2 = 90 degree X pulse
      jxh = X-H coupling constant
      dpwr2 = power level for X decoupling
      tpwr = power level for H pulses
      pw = 90 degree H pulse
      phase = 1,2: gives HYPERCOMPLEX (t1) acquisition;
      ni = number of t1 increments
      phase2 = 1,2: gives HYPERCOMPLEX (t2) acquisition;
      ni2 = number of t2 increments
      satflg = flag to do xmtr presaturation at satfrq and satpwr
               during relaxation (satdly) and mixing (mix) periods
      satfrq = saturation frequency for xmtr presaturation
      satpwr = saturation power for xmtr presaturation
      satdly = saturation period follows D1
      sspul = 'y': selects for saturation sequence at start of pulse sequence
      nosyflg = 'n': turns off NOESY part of the sequence
      hmqcflg = 'n': turns off HMQC part of the sequence

Plane processing:

      array = phase2,phase

      np x ni : wft2d('ni',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0)
      np x ni2 : wft2d('ni2',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0)

      array = phase,phase2

      np xni : wft2d('ni',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0)
      np x ni2 : wft2d('ni2',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0)

phase table:

      t1 = 0 2 0 2 1 3 1 3 - first proton pulse
      t2 = 0 0 2 2 1 1 3 3 - first carbon pulse
      t3 = 0 0 0 0 1 1 1 1 - all other pulses
      t4 = 0 2 2 0 1 3 3 1 - receiver
      t5 = 0 2 0 2 1 3 1 3 - receiver if hmqcflg='n'

      Feb 07 1991 (vvk)
revised: March 01, 1991 (vvk) June 14, 1991(gg)
      Sept 29, 1991 (bds/cms) to correct satflg[C] */

#include <standard.h>

pulsesequance()
{
      double      satpwr,
                  satdly,
                  satfrq,
                  mix,
                  jxh,
                  pwx2lvl,
                  pwx2,

```

```

        int          tau;
                    phase2,
                    phase;
        char          sspul[MAXSTR],
                    satflg[MAXSTR],
                    nosyflg[MAXSTR],
                    hmqcflg[MAXSTR];

/* LOAD VARIABLES */
satdly = getval("satdly");
satfrq = getval("satfrq");
satpwr = getval("satpwr");
mix = getval("mix");
pwx2lv1 = getval("pwx2lv1");
pwx2 = getval("pwx2");
jxh = getval("jxh");
tau = 1/(2.0*jxh);
phase = (int) (getval("phase") + 0.5);
phase2 = (int) (getval("phase2") + 0.5);
getstr("sspul", sspul);
getstr("satflg", satflg);
getstr("nosyflg", nosyflg);
getstr("hmqcflg", hmqcflg);

initval(pwx2lv1, v6);
initval(dpwr2, v7);
initval(tpwr, v8);
initval(satpwr, v9);

/* CHECK CONDITIONS */

/* DETERMINE STEADY-STATE MODE */

/* STEADY-STATE PHASECYCLING */

/* PHASECYCLE */
loadtable("nosyhmqc3d"); /* t1 = 0 2 0 2 1 3 1 3
                           t2 = 0 0 2 2 1 1 3 3
                           t3 = 0 0 0 0 1 1 1 1
                           t4 = 0 2 2 0 1 3 3 1
                           t5 = 0 2 0 2 1 3 1 3 */

getelem(t1, ct, v1);
getelem(t2, ct, v2);

if (hmqcflg[A] == 'n')
    getelem(t5, ct, oph);
else
    getelem(t4, ct, oph);

initval(2.0*(double) (((int) (d2*getval("sw1")+0.5)%2)), v13);
initval(2.0*(double) (((int) (d3*getval("sw2")+0.5)%2)), v14);

    if (phase == 2)
        incr(v1);
    if (phase2 == 2)
        incr(v2);
    add(v1, v13, v1);
    add(v2, v14, v2);

add(oph, v13, oph);
add(oph, v14, oph);

/* BEGIN THE ACTUAL PULSE SEQUENCE */
status(A); /* relaxation status */
power(v8, TODEV);
power(v7, DO2DEV);
dec2phase(zero);
if (sspul[A] == 'y')
[
    rgpulse(pw*200, zero, rof1, rof2);

```

```

    rgpulse(pw*200, one, rof1,rof2);
}
else
    hsdelay(d1);

if (satflg[A] == 'y')
{
    if (satfrq != tof)
        offset(satfrq,TODEV);
    power(v9,TODEV);
    rgpulse(satdly,zero,rof1,rof1);
    power(v8,TODEV);
    if (satfrq != tof)
        offset(tof,TODEV);
    delay(40.0e-6);
}
else
    delay(satdly);

if (nosyflg[A] != 'n')
{
    rgpulse(pw, v1, rof1, 1.0e-6);
}

status(B);
if (d2>0)
    delay(d2 - rof1 - 1.0e-6 - (4*pw/3.1416));
else delay(d2);

status(C);
rgpulse(pw, t3, rof1, 1.0e-6);
if (satflg[C] == 'y')
{
    if (satfrq != tof)
        offset(satfrq,TODEV);
    power(v9,TODEV);
    rgpulse(mix,zero,2.0e-6,rof1);
    power(v8,TODEV);
    if (satfrq != tof)
        offset(tof,TODEV);
    delay(40.0e-6);
}
else
    hsdelay(mix);
status(D);
power(v6,DO2DEV);
rgpulse(pw, t3, rof1, 0.0);
}
else
    rgpulse(pw, v1, rof1, 0.0);

if (hmqcflg[A] != 'n')
{
    rcvloff();
    delay(tau - 0.5*pw - 0.5*px2 - rof1);
    txphase(t3);
    dec2rgpulse(pwx2,v2,rof1,0.0);
    dec2phase(t3);
    delay(d3/2.0);
    rgpulse(2.0*pw, t3, 0.0, 0.0);
    dec2phase(t3);
    delay(d3/2.0);
    dec2rgpulse(pwx2,t3,0.0,rof1);
    rcvtron();
    delay(tau - 4.2e-6 - 0.5*px2 - rof1);
}
power(v7,DO2DEV);
delay(rof2);

status(E);
/* acquisition status */
}

```

6. <sup>15</sup>N-edited TOCSY HMQC.

```

/* tocsyhmqc3d3rf - TOCSY-HMQC 3D sequence
- written in hypercomplex phase sensitive mode only
- uses the 2nd decoupler for the X pulses
- modified from nosyhmqc3d3rf by smg/bds Feb. 20 1992
- last revised Feb. 24 1992 by smg

Sequence:

TOCSY-HMQC:

status : A-|--B--|---C---|-----D-----|---E---
1H : -90-t1-spinlock-1/2J- 180 -1/2J- Acq (t3)
X : BB BB 90-t2/2- -t2/2-90 BB
phtable : t1 t5 t2 t3 t3 t4

Parameters:

d2 = First evolution time (TOCSY)
d3 = Second evolution time (HMQC)
pwx2lvl = power level for X pulses
pwx2 = 90 degree X pulse
jxh = X-H coupling constant
dpwr2 = power level for X decoupling
tpwr = power level for H pulses
pw = 90 degree H pulse at power tpwr
phase = 1,2: gives HYPERCOMPLEX (t1) acquisition;
ni = number of t1 increments
phase2 = 1,2: gives HYPERCOMPLEX (t2) acquisition;
ni2 = number of t2 increments
satflg = flag to do xmtr presaturation at satfrq and satpwr
during relaxation (satdly)
satfrq = saturation frequency for xmtr presaturation
satpwr = saturation power for xmtr presaturation
satdly = saturation period follows D1
p1lvl = power level for spinlock
p1 = 90 degree H pulse for mlev17
mix = TOCSY mixing time
trim = trim pulse preceeding mlev17
hs = 'yn': homospoil pulse (hst) during the d1 relaxation delay
wdwfctr = multiplication 'window' factor of p1

Plane processing:

array = phase2,phase

np x ni : wft2d('ni',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0,0)
np x ni2 : wft2d('ni2',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0,0)

array = phase,phase2

np xni : wft2d('ni',#i,1,0,0,0,0,0,0,0,0,0,0,-1,0,0,0,0)
np x ni2 : wft2d('ni2',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0,0)

phase table:

t1 = 0 0 1 1 - first proton pulse
t2 = 0 2 1 3 - first nitrogen pulse
t3 = 0 0 1 1 - 180 and second nitrogen pulse
t4 = 0 2 1 3 - receiver
t5 = 0 0 1 1 2 2 3 3 - spinlock

modified by smg/bds 20 Feb 92 */

#include <standard.h>

mleva()
{
double wdwfctr>window;
wdwfctr=getval(*wdwfctr*);
window = (wdwfctr*p1);
rgpulse(p1,v3,0.0>window);
rgpulse(2*p1,v4,0.0>window);
rgpulse(p1,v3,0.0,0.0);
}

```

```

mlevb()
{
    double wdwfctr,window;
    wdwfctr=getval("wdwfctr");
    window = (wdwfctr*p1);
    rgpulse(p1,v5,0.0,window);
    rgpulse(2*p1,v12,0.0,window);
    rgpulse(p1,v5,0.0,0.0);
}

pulsesequenece()
{
    double
        satpwr,
        satdly,
        satfrq,
        mix,
        jxh,
        pwx2lv1,
        pwx2,
        cycles,
        trim,
        wdwfctr,
        window,
        pllvl,
        tau;
    int
        phase,
        phase2;
    char
        satflg[MAXSTR];

/* LOAD VARIABLES */
    satdly = getval("satdly");
    satfrq = getval("satfrq");
    satpwr = getval("satpwr");
    pllvl = getval("pllvl");
    mix = getval("mix");
    trim = getval("trim");
    wdwfctr = getval("wdwfctr");
    window = (wdwfctr*p1);
    pwx2lv1 = getval("pwx2lv1");
    pwx2 = getval("pwx2");
    jxh = getval("jxh");
    tau = 1/(2.0*jxh);
    phase = (int) (getval("phase") + 0.5);
    phase2 = (int) (getval("phase2") + 0.5);
    getstr("satflg",satflg);

    initval(pwx2lv1,v6);
    initval(dpwr2,v7);
    initval(tpwr,v8);
    initval(satpwr,v9);
    initval(pllvl,v10);

/* CHECK CONDITIONS */
    if ((pllvl-tpwr) >=0)
    {
        printf("tpwr is for H hard pulse. \n");
        abort(1);
    }

/* DETERMINE STEADY-STATE MODE */

/* STEADY-STATE PHASECYCLING

/* PHASECYCLE */
    loadtable("toxyhmqc3d"); /* t1 = 0 0 1 1
                                t2 = 0 2 1 3
                                t3 = 0 0 1 1
                                t4 = 0 2 1 3
                                t5 = 0 0 1 1 2 2 3 3
                                */

```



```

getelem(t1,ct,v1);
getelem(t2,ct,v2);
getelem(t4,ct,oph);
getelem(t5,ct,v3);

initval(2.0*(double) (((int) (d2*getval("sw1")+0.5)%2)),v13);
initval(2.0*(double) (((int) (d3*getval("sw2")+0.5)%2)),v14);

if (phase == 2)
    incr(v1);
if (phase2 == 2)
    incr(v2);

add(v1,v13,v1);
add(v2,v14,v2);

add(one,v3,v4);
add(one,v4,v5);
add(one,v5,v12);

add(oph,v13,oph);
add(oph,v14,oph);

/* BEGIN THE ACTUAL PULSE SEQUENCE */
status(A);
power(v8,TODEV); /* preparation period */
power(v7,DO2DEV);
dec2phase(zero);
hsdelay(d1);

if (satflg[A] == 'y')
{
    if (satfrq != tof)
        offset(satfrq,TODEV);
    power(v9,TODEV);
    rgpulse(satdly,zero,rofl,rofl);
    power(v8,TODEV);
    if (satfrq != tof)
        offset(tof,TODEV);
    delay(40.0e-6);
}
else
    delay(satdly);

status(B);
rgpulse(pw,v1,rofl,1.0e-6); /* t1 evolution period */
power(v10,TODEV);
rcvroff();

txphase(v4);
if (d2>0)
    delay(d2 - 1.0e-6 -5.0e-6 -(2*pw/3.1416));
else delay(d2);

status(C);
/* calculate and initialize loop counter */
/* TOCSY mixing period */
cycles = (mix-trim)/(64.66*p1+32*window);
cycles=2.0*(double)(int)(cycles/2.0);
initval(cycles,v11);
if (cycles > 1.0)
{
    rgpulse(trim,v4,5.0e-6,0.0);
    starthardloop(v11);
    mleva(); mlevb(); mlevb(); mleva();
    mlevb(); mlevb(); mleva(); mleva();
    mlevb(); mleva(); mleva(); mlevb();
    mleva(); mleva(); mlevb(); mlevb();
    rgpulse(0.66*p1,v4,0.0,0.0);
    endhardloop();
}

status(D);
power(v8,TODEV); /* HMQC and t2 evolution period */
power(v6,DO2DEV);
rcvroff();

```

```

delay(tau - 0.5*px2 - rof1);
txphase(t3);
dec2rgpulse(px2,v2,rof1,0.0);
dec2phase(t3);
delay(d3/2.0);
rgpulse(2.0*pw, t3, 0.0, 0.0);
dec2phase(t3);
delay(d3/2.0);
dec2rgpulse(px2,t3,0.0,rof1);
rcvtron();
delay(tau - 4.2e-6 - 0.5*px2 - rof1);
power(v7,DO2DEV);
delay(rof2);

status(E); /* acquisition status */
}

```

7. <sup>13</sup>C-edited NOESY HMQC.

```

/* nosyhmqc3d - NOESY-HMQC 3D sequence
   Will do standard HMQC, standard NOESY, or NOESY-HMQC 3D
   - written in hypercomplex phase sensitive mode only

Sequence:

NOESY-HMQC:
status : A-[-B]----C-----|-----D-----|-----E-----
1H : 90-t1-90-mix-90-1/2J- 180 -1/2J- Acq (t3)
X : BB 90-t2/2- -t2/2-90 BB
phase : t1 t3 t3 t2 t3 t3 t4 or t5

Parameters:

d2 = First evolution time
d3 = Second evolution time
mix = NOESY mixing time.
pwxlvl = power level for X pulses
pwx = 90 degree X pulse
jxh = X-H coupling constant
dpwr = power level for X decoupling
tpwr = power level for H pulses
pw = 90 degree H pulse
phase = 1,2: gives HYPERCOMPLEX (t1) acquisition;
ni1 = number of t1 increments
phase2 = 1,2: gives HYPERCOMPLEX (t2) acquisition;
ni2 = number of t2 increments

satflg = flag to do xtmr presaturation at satfrq and satpwr
         during relaxation(satdly) and mixing(mix) periods [ynyn]
satfrq = saturation frequency for xtmr presaturation
satpwr = saturation power for xtmr presaturation
satdly = saturation period follows d1
sspul = 'y': selects for HS-90-HS sequence at start of pulse sequence
nosyflg= 'n': turns off NOESY part of the sequence (std HMQC with the current
               phase cycling)
hmqcflg= 'n': turns off HMQC part of the sequence (std NOESY with only 2-step
               phase cycling)

plane processing:

array = phase2,phase

np x ni1 : wft2d('ni1',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0,0)
np x ni2 : wft2d('ni2',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0)

array = phase, phase2

np x ni1 : wft2d('ni1',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0)
np x ni2 : wft2d('ni2',#i,1,0,0,0,0,0,0,0,0,0,-1,0,0,0,0)

phase table:

t1 = 0 2 0 2 1 3 1 3 - first H pulse
t2 = 0 0 2 2 1 1 3 3 - first X pulse
t3 = 0 0 0 0 1 1 1 1 - all other pulses
t4 = 0 2 2 0 1 3 3 1 - receiver
t5 = 0 2 0 2 1 3 1 3 - receiver if hmqcflg = 'n'

Feb 07 1991 (vvk)
revised: March 01, 1991 (vvk) typed in bds 4 April 91

revised: November 05, 1992 by S. Gagne ( change status C to
mixing time only)

revised: May 02, 1993 by S. Gagne : change HMQC part to be
like 2D hmqc (so now uses decon/decoff... during HMQC
portion). See 13C excitation profile in 'Pulse Sequence
Notes' p. 7-8.

```

\*/

```

#include <standard.h>

pulsesequance()
{
    double          satpwr,
                   satdly,
                   satfrq,
                   mix,
                   jxh,
                   pwxlv1,
                   pwx,
                   tau;
    int             phase2,
                   phase;
    char            sspul[MAXSTR],
                   satflg[MAXSTR],
                   nosyflg[MAXSTR],
                   hmqcflg[MAXSTR];

/* LOAD VARIABLES */
satdly = getval("satdly");
satfrq = getval("satfrq");
satpwr = getval("satpwr");
mix = getval("mix");
pwxlv1 = getval("pwxlv1");
pwx = getval("pwx");
jxh = getval("jxh");
tau = 1/(2.0*jxh);
phase = (int) (getval("phase") + 0.5);
phase2 = (int) (getval("phase2") + 0.5);
getstr("sspul", sspul);
getstr("satflg", satflg);
getstr("nosyflg", nosyflg);
getstr("hmqcflg", hmqcflg);

    initval(pwxlv1, v6);
    initval(dpwr, v7);
    initval(tpwr, v8);
    initval(satpwr, v9);

/* CHECK CONDITIONS */

/* DETERMINE STEADY-STATE MODE */

/* STEADY-STATE PHASECYCLING */
/* PHASECYCLE */
    loadtable("nosyhmqc3d");
    getelem(t1, ct, v1);
    getelem(t2, ct, v2);

    if (hmqcflg[A] == 'n')
        getelem(t5, ct, oph);
    else
        getelem(t4, ct, oph);

    initval(2.0*(double) (((int) (d2*getval("sw1")+0.5)%2)), v13);
    initval(2.0*(double) (((int) (d3*getval("sw2")+0.5)%2)), v14);

    if (phase == 2)
        incr(v1);
    if (phase2 == 2)
        incr(v2);
    add(v1, v13, v1);
    add(v2, v14, v2);

    add(oph, v13, oph);
    add(oph, v14, oph);

/* BEGIN THE ACTUAL PULSE SEQUENCE */

```

```

status(A);                                /* relaxation status */
power(v8,TODEV);
power(v7,DODEV);
decphase(zero);
if (sspul[A] == 'y')
{
    hsdelay(hst + 0.001);
    rgpulse(pw, zero, rof1,rof2);
    hsdelay(hst+d1);
}
else
    hsdelay(d1);
if (satflg[A] == 'y')
{
    if (satfrq != tof)
        offset(satfrq,TODEV);
    power(v9,TODEV);
    rgpulse(satdly,zero,rof1,rof1);
    power(v8,TODEV);
    if (satfrq != tof)
        offset(tof,TODEV);
    delay(40.0e-6);
}
else
    delay(satdly);

if (nosyflg[A] != 'n')
{
    rgpulse(pw, v1, rof1, 1.0e-6);
}

status(B);
if (d2>0)
    delay(d2 - rof1 - 1.0e-6 - (4*pw/3.1416));
else delay(d2);

status(C);
rgpulse(pw, t3, rof1, 1.0e-6);
if (satflg[C] == 'y')
{
    if (satfrq != tof)
        offset(satfrq,TODEV);
    power(v9,TODEV);
    rgpulse(mix,zero,2.0e-6,rof1);
    power(v8,TODEV);
    if (satfrq != tof)
        offset(tof,TODEV);
    delay(40.0e-6);
}
else
    hsdelay(mix);
status(D);
power(v6,DODEV);
rgpulse(pw, t3, rof1, 0.0);
}
else
    rgpulse(pw, v1, rof1, 0.0);

if (hmqcflg[A] != 'n')
{
    rcvroff();
    txphase(t3);
    decphase(v2);
    delay(tau - 0.5*pw - 0.5*px - rof1);
    if (d3 <= 2*pw) /* allows 1H 180 to occur during */
    { /* t1 evolution period */
        delay(rof1);
        if (px >= (pw - 0.5e-6))
        {
            decon();
            delay(px - (2*pw - d3)/2);
            xmtron();
            delay(pw - d3/2);
            decoff();
            decphase(t3);
            delay(d3);
        }
    }
}

```

```

        decon();
        delay(pw - d3/2);
        xmtroff();
        delay(pwx - (2*pw - d3)/2);
        decoff();
    }
    else
    {
        xmtron();
        delay((2*pw - d3)/2 - pwx);
        decon();
        delay(pwx);
        decoff();
        decphase(t3);
        delay(d3);
        decon();
        delay(pwx);
        decoff();
        delay((2*pw - d3)/2 - pwx);
        xmtroff();
    }
}
else
{
    decrgpulse(pwx,v2,rof1,0.0);
    delay(d3/2.0 - pw + 0.5e-6);
    decphase(t3);
    rgpulse(2.0*pw, t3, 0.0, 0.0);
    delay(d3/2.0 - pw + 0.5e-6);
    decrgpulse(pwx,t3,0.0,0.0);
}
rcvtron();
    delay(rof2);
power(v7,DODEV);
    delay(tau - 4.2e-6 - 0.5*pwx - rof2);
}
status(E);
/* acquisition status */
}

```

APPENDIX **B****ASSIGNMENTS**

|       |         |
|-------|---------|
| 1 ALA |         |
| CA    | 51.760  |
| HA    | 4.140   |
| CB    | 19.300  |
| HB#   | 1.560   |
| C     | 174.200 |
| ; 0.0 |         |
| 2 SER |         |
| CA    | 58.310  |
| HA    | 4.520   |
| CB    | 63.800  |
| HB#   | 3.880   |
| C     | 173.900 |
| ; 0.0 |         |
| 3 MET |         |
| #N    |         |
| #HN   |         |
| CA    | 55.950  |
| HA    | 4.640   |
| #CB   |         |
| #HB1  |         |
| #HB2  |         |
| #CG   |         |
| #HG1  |         |
| #HG2  |         |
| CE    | 16.600  |
| HE#   | 2.070   |
| C     | 176.600 |
| ; 0.0 |         |
| 4 THR |         |
| N     | 116.090 |
| HN    | 8.013   |
| CA    | 61.085  |
| HA    | 4.437   |
| CB    | 70.400  |
| HB    | 4.652   |
| CG2   | 21.310  |
| HG2#  | 1.176   |
| C     | 175.000 |
| ; 0.0 |         |
| 5 ASP |         |
| N     | 123.048 |
| HN    | 8.689   |
| CA    | 57.323  |
| HA    | 4.443   |
| CB    | 40.195  |
| HB1   | 2.627   |
| HB2   | 2.733   |
| CG    | 179.800 |
| C     | 178.700 |
| ; 0.0 |         |
| 6 GLN |         |
| N     | 120.857 |
| HN    | 8.869   |
| CA    | 58.810  |
| HA    | 4.066   |
| CB    | 30.575  |
| HB1   | 2.050   |
| HB2   | 2.166   |
| CG    | 35.403  |
| HG1   | 2.345   |
| HG2   | 2.500   |

|        |         |
|--------|---------|
| CD     | 180.300 |
| NE2    | 112.861 |
| HE21   | 7.088   |
| HE22   | 7.292   |
| C      | 179.100 |
| ; 0.0  |         |
| 7 GLN  |         |
| N      | 123.005 |
| HN     | 7.830   |
| CA     | 58.260  |
| HA     | 3.927   |
| #CB    |         |
| HB1    | 2.080   |
| HB2    | 2.170   |
| CG     | 33.600  |
| HG#    | 2.420   |
| CD     | 180.300 |
| NE2    | 112.676 |
| HE21   | 7.238   |
| HE22   | 7.552   |
| C      | 177.800 |
| ; 0.0  |         |
| 8 ALA  |         |
| N      | 124.412 |
| HN     | 8.502   |
| CA     | 54.890  |
| HA     | 4.137   |
| CB     | 17.930  |
| HB#    | 1.480   |
| C      | 181.100 |
| ; 0.0  |         |
| 9 GLU  |         |
| N      | 120.466 |
| HN     | 8.130   |
| CA     | 58.800  |
| HA     | 4.043   |
| CB     | 29.800  |
| HB#    | 2.070   |
| #CG    |         |
| HG1    | 2.190   |
| HG2    | 2.380   |
| CD     | 180.000 |
| C      | 178.800 |
| ; 0.0  |         |
| 10 ALA |         |
| N      | 124.523 |
| HN     | 7.918   |
| CA     | 55.467  |
| HA     | 4.112   |
| CB     | 18.007  |
| HB#    | 1.405   |
| C      | 179.200 |
| ; 0.0  |         |
| 11 ARG |         |
| N      | 116.476 |
| HN     | 7.965   |
| CA     | 59.390  |
| HA     | 3.850   |
| CB     | 29.980  |
| HB#    | 1.820   |
| CG     | 26.370  |
| HG1    | 1.660   |



|        |         |
|--------|---------|
| HG2    | 1.720   |
| CD     | 43.100  |
| HD#    | 3.240   |
| #NE    |         |
| #HE    |         |
| #NH1   |         |
| #HH11  |         |
| #HH12  |         |
| #NH2   |         |
| #HH21  |         |
| #HH22  |         |
| C      | 178.000 |
| ; 0.0  |         |
| 12 ALA |         |
| N      | 120.407 |
| HN     | 7.687   |
| CA     | 53.670  |
| HA     | 4.230   |
| CB     | 18.500  |
| HB#    | 1.460   |
| C      | 178.600 |
| ; 0.0  |         |
| 13 PHE |         |
| N      | 119.369 |
| HN     | 7.685   |
| CA     | 59.243  |
| HA     | 4.383   |
| CB     | 41.017  |
| HB1    | 3.225   |
| HB2    | 3.280   |
| #CD#   |         |
| #HD#   | 7.270   |
| HD1    | 7.270   |
| HD2    | 7.270   |
| #CE#   |         |
| #HE#   | 7.380   |
| HE1    | 7.380   |
| HE2    | 7.380   |
| #CZ    |         |
| #HZ    |         |
| C      | 175.700 |
| ; 0.0  |         |
| 14 LEU |         |
| N      | 119.357 |
| HN     | 7.366   |
| CA     | 53.588  |
| HA     | 4.498   |
| CB     | 44.700  |
| HB1    | 1.508   |
| HB2    | 1.932   |
| CG     | 26.490  |
| HG     | 2.106   |
| CD1    | 28.310  |
| HD1#   | 0.925   |
| CD2    | 24.542  |
| HD2#   | 1.126   |
| C      | 176.000 |
| ; 0.0  |         |
| 15 SER |         |
| N      | 117.230 |
| HN     | 7.832   |
| CA     | 56.340  |
| HA     | 4.586   |
| CB     | 65.378  |
| HB1    | 4.036   |
| HB2    | 4.424   |
| C      | 175.000 |
| ; 0.0  |         |
| 16 GLU |         |
| N      | 122.712 |
| HN     | 9.048   |
| CA     | 60.087  |
| HA     | 3.973   |
| CB     | 29.200  |
| HB1    | 2.035   |
| HB2    | 2.070   |
| CG     | 36.780  |
| HG1    | 2.353   |

|        |         |
|--------|---------|
| HG2    | 2.413   |
| CD     | 183.700 |
| C      | 179.600 |
| ; 0.0  |         |
| 17 GLU |         |
| N      | 120.911 |
| HN     | 8.887   |
| CA     | 60.227  |
| HA     | 4.072   |
| CB     | 28.833  |
| HB1    | 1.942   |
| HB2    | 2.047   |
| CG     | 36.700  |
| HG1    | 2.295   |
| HG2    | 2.370   |
| CD     | 184.400 |
| C      | 179.100 |
| ; 0.0  |         |
| 18 MET |         |
| N      | 121.593 |
| HN     | 7.730   |
| CA     | 58.345  |
| HA     | 4.034   |
| CB     | 33.162  |
| HB1    | 1.852   |
| HB2    | 2.113   |
| CG     | 32.673  |
| HG1    | 2.413   |
| HG2    | 2.468   |
| CE     | 17.340  |
| HE#    | 2.030   |
| C      | 178.100 |
| ; 0.0  |         |
| 19 ILE |         |
| N      | 121.192 |
| HN     | 8.243   |
| CA     | 66.778  |
| HA     | 3.717   |
| CB     | 37.744  |
| HB     | 1.994   |
| CG1    | 29.100  |
| HG11   | 1.045   |
| HG12   | 1.700   |
| CD1    | 13.400  |
| HD1#   | 0.920   |
| CG2    | 17.453  |
| HG2#   | 1.206   |
| C      | 177.600 |
| ; 0.0  |         |
| 20 ALA |         |
| N      | 122.221 |
| HN     | 7.842   |
| CA     | 55.120  |
| HA     | 4.193   |
| CB     | 17.700  |
| HB#    | 1.510   |
| C      | 181.300 |
| ; 0.0  |         |
| 21 GLU |         |
| N      | 122.218 |
| HN     | 7.769   |
| CA     | 59.150  |
| HA     | 4.140   |
| CB     | 29.200  |
| HB1    | 1.965   |
| HB2    | 2.050   |
| CG     | 36.100  |
| HG1    | 2.180   |
| HG2    | 2.410   |
| CD     | 183.700 |
| C      | 180.300 |
| ; 0.0  |         |
| 22 PHE |         |
| N      | 121.178 |
| HN     | 8.720   |
| CA     | 58.800  |
| HA     | 5.027   |
| CB     | 37.660  |

|        |         |        |         |
|--------|---------|--------|---------|
| HB#    | 3.510   | N      | 120.330 |
| #CD#   |         | HN     | 7.305   |
| #HD#   | 7.200   | CA     | 57.880  |
| HD1    | 7.190   | HA     | 4.055   |
| HD2    | 7.190   | CB     | 32.007  |
| #CE#   |         | HB1    | 2.058   |
| #HE#   | 7.340   | HB2    | 2.158   |
| HE1    | 7.340   | CG     | 33.030  |
| HE2    | 7.340   | HG1    | 2.408   |
| #CZ    |         | HG2    | 2.625   |
| #HZ    |         | CE     | 17.527  |
| C      | 178.500 | HE#    | 1.962   |
| ; 0.0  |         | C      | 177.900 |
| 23 LYS |         | ; 0.0  |         |
| N      | 125.141 | 29 PHE |         |
| HN     | 9.240   | N      | 119.395 |
| CA     | 58.280  | HN     | 7.777   |
| HA     | 3.953   | CA     | 58.213  |
| CB     | 31.315  | HA     | 4.304   |
| HB#    | 1.992   | CB     | 38.930  |
| CG     | 24.130  | HB1    | 2.768   |
| HG#    | 1.091   | HB2    | 2.897   |
| CD     | 26.754  | #CD#   |         |
| HD1    | 0.185   | #HD#   | 7.160   |
| HD2    | 1.371   | HD1    | 7.170   |
| CE     | 42.003  | HD2    | 7.170   |
| HE#    | 2.605   | #CE#   |         |
| C      | 177.200 | #HE#   |         |
| ; 0.0  |         | #CZ    |         |
| 24 ALA |         | #HZ    |         |
| N      | 122.246 | C      | 177.700 |
| HN     | 7.570   | ; 0.0  |         |
| CA     | 54.910  | 30 ASP |         |
| HA     | 4.190   | N      | 119.737 |
| CB     | 17.700  | HN     | 8.084   |
| HB#    | 1.505   | CA     | 52.053  |
| C      | 180.700 | HA     | 4.504   |
| ; 0.0  |         | CB     | 38.748  |
| 25 ALA |         | HB1    | 1.462   |
| N      | 122.034 | HB2    | 2.469   |
| HN     | 7.572   | CG     | 178.800 |
| CA     | 55.050  | C      | 176.700 |
| HA     | 3.980   | ; 0.0  |         |
| CB     | 18.422  | 31 ALA |         |
| HB#    | 1.724   | N      | 130.390 |
| C      | 177.900 | HN     | 7.682   |
| ; 0.0  |         | CA     | 54.985  |
| 26 PHE |         | HA     | 4.110   |
| N      | 121.157 | CB     | 19.330  |
| HN     | 8.882   | HB#    | 1.534   |
| CA     | 62.218  | C      | 179.100 |
| HA     | 3.170   | ; 0.0  |         |
| CB     | 39.600  | 32 ASP |         |
| HB#    | 2.740   | N      | 114.340 |
| #CD#   |         | HN     | 8.193   |
| #HD#   | 6.680   | CA     | 52.727  |
| HD1    | 6.680   | HA     | 4.592   |
| HD2    | 6.680   | CB     | 40.000  |
| #CE1   |         | HB1    | 2.732   |
| #HE#   | 7.100   | HB2    | 3.110   |
| HE1    | 7.100   | CG     | 181.800 |
| HE2    | 7.100   | C      | 177.800 |
| #CZ    |         | ; 0.0  |         |
| #HZ    |         | 33 GLY |         |
| C      | 176.600 | N      | 111.810 |
| ; 0.0  |         | HN     | 8.020   |
| 27 ASP |         | CA     | 46.700  |
| N      | 118.589 | HA#    | 3.850   |
| HN     | 8.571   | C      | 175.700 |
| CA     | 56.900  | ; 0.0  |         |
| HA     | 4.186   | 34 GLY |         |
| CB     | 40.005  | N      | 109.600 |
| HB1    | 2.613   | HN     | 8.137   |
| HB2    | 2.670   | CA     | 46.300  |
| CG     | 179.800 | HA1    | 3.980   |
| C      | 177.800 | HA2    | 4.090   |
| ; 0.0  |         | C      | 175.900 |
| 28 MET |         | ; 0.0  |         |

|        |         |
|--------|---------|
| 35 GLY |         |
| N      | 113.800 |
| HN     | 10.685  |
| CA     | 45.255  |
| HA1    | 3.673   |
| HA2    | 4.475   |
| C      | 173.200 |
| ; 0.0  |         |
| 36 ASP |         |
| N      | 116.198 |
| HN     | 7.750   |
| CA     | 52.568  |
| HA     | 5.175   |
| CB     | 41.385  |
| HB1    | 3.053   |
| HB2    | 2.262   |
| CG     | 179.400 |
| C      | 173.100 |
| ; 0.0  |         |
| 37 ILE |         |
| N      | 125.902 |
| HN     | 9.613   |
| CA     | 60.082  |
| HA     | 4.942   |
| CB     | 39.935  |
| HB     | 1.821   |
| CG1    | 26.997  |
| HG1#   | 1.200   |
| CD1    | 14.739  |
| HD1#   | 0.475   |
| CG2    | 17.555  |
| HG2#   | 0.941   |
| C      | 175.700 |
| ; 0.0  |         |
| 38 SER |         |
| N      | 124.028 |
| HN     | 8.696   |
| CA     | 55.672  |
| HA     | 4.848   |
| CB     | 66.547  |
| HB1    | 3.990   |
| HB2    | 4.490   |
| C      | 175.900 |
| ; 0.0  |         |
| 39 THP |         |
| N      | 115.113 |
| HN     | 9.216   |
| CA     | 66.900  |
| HA     | 3.822   |
| CB     | 68.243  |
| HB     | 4.200   |
| CG2    | 23.170  |
| HG2#   | 1.370   |
| C      | 177.000 |
| ; 0.0  |         |
| 40 LYS |         |
| N      | 122.682 |
| HN     | 7.840   |
| CA     | 59.257  |
| HA     | 4.120   |
| CB     | 32.630  |
| HB1    | 1.775   |
| HB2    | 1.923   |
| CG     | 24.743  |
| HG1    | 1.407   |
| HG2    | 1.500   |
| CD     | 29.235  |
| HD#    | 1.683   |
| CE     | 42.013  |
| HE#    | 2.995   |
| C      | 179.700 |
| ; 0.0  |         |
| 41 GLU |         |
| N      | 122.287 |
| HN     | 7.698   |
| CA     | 58.795  |
| HA     | 4.130   |
| #CB    |         |

|        |         |
|--------|---------|
| #HB1   |         |
| #HB2   |         |
| CG     | 35.800  |
| HG#    | 2.473   |
| CD     | 180.000 |
| C      | 179.700 |
| ; 0.0  |         |
| 42 LEU |         |
| N      | 122.373 |
| HN     | 8.519   |
| CA     | 57.820  |
| HA     | 3.990   |
| CB     | 42.894  |
| HB1    | 1.607   |
| HB2    | 1.762   |
| CG     | 26.525  |
| HG     | 1.630   |
| CD1    | 25.520  |
| HD1#   | 0.821   |
| CD2    | 24.130  |
| HD2#   | 0.840   |
| C      | 178.700 |
| ; 0.0  |         |
| 43 GLY |         |
| N      | 107.127 |
| HN     | 8.584   |
| CA     | 47.795  |
| HA1    | 3.592   |
| HA2    | 3.975   |
| C      | 175.500 |
| ; 0.0  |         |
| 44 THR |         |
| N      | 119.744 |
| HN     | 7.942   |
| CA     | 67.195  |
| HA     | 3.935   |
| CB     | 69.040  |
| HB     | 4.351   |
| CG2    | 21.675  |
| HG2#   | 1.323   |
| C      | 176.300 |
| ; 0.0  |         |
| 45 VAL |         |
| N      | 121.999 |
| HN     | 7.298   |
| CA     | 66.310  |
| HA     | 3.687   |
| CB     | 31.360  |
| HB     | 1.993   |
| CG1    | 22.200  |
| HG1#   | 0.815   |
| CG2    | 21.200  |
| HG2#   | 0.673   |
| C      | 177.500 |
| ; 0.0  |         |
| 46 MET |         |
| N      | 117.661 |
| HN     | 8.006   |
| CA     | 59.364  |
| HA     | 3.910   |
| CB     | 32.070  |
| HB1    | 1.818   |
| HB2    | 2.068   |
| CG     | 33.881  |
| HG1    | 2.435   |
| HG2    | 2.636   |
| CE     | 17.527  |
| HE#    | 1.932   |
| C      | 178.800 |
| ; 0.0  |         |
| 47 ARG |         |
| N      | 120.507 |
| HN     | 8.218   |
| CA     | 58.900  |
| HA     | 4.643   |
| CB     | 30.100  |
| HB1    | 1.930   |
| HB2    | 1.990   |

|        |         |        |         |
|--------|---------|--------|---------|
| CG     | 28.497  | CG     | 178.100 |
| HG1    | 1.810   | ND2    | 113.622 |
| HG2    | 1.910   | HD21   | 6.770   |
| CD     | 43.200  | HD22   | 7.520   |
| HD#    | 3.246   | C      | 172.100 |
| NE     | 86.000  | ; 0.0  |         |
| HE     | 7.430   | 53 PRO |         |
| #NH1   |         | #N     |         |
| #HH11  |         | CA     | 62.553  |
| #HH12  |         | HA     | 4.744   |
| #NH2   |         | CB     | 32.058  |
| #HH21  |         | HB1    | 1.930   |
| #HH22  |         | HB2    | 2.231   |
| C      | 181.000 | #CG    |         |
| ; 0.0  |         | HG#    | 1.990   |
| 48 MET |         | CD     | 49.925  |
| N      | 122.785 | HD1    | 3.240   |
| HN     | 7.940   | HD2    | 3.649   |
| CA     | 58.803  | C      | 177.800 |
| HA     | 4.221   | ; 0.0  |         |
| CB     | 32.636  | 54 THR |         |
| HB1    | 2.324   | N      | 114.323 |
| HB2    | 2.538   | HN     | 8.600   |
| CG     | 32.986  | CA     | 60.233  |
| HG1    | 2.702   | HA     | 4.428   |
| HG2    | 2.935   | CB     | 71.525  |
| CE     | 17.515  | HB     | 4.730   |
| HE#    | 2.152   | CG2    | 21.860  |
| C      | 175.600 | HG2#   | 1.355   |
| ; 0.0  |         | C      | 175.200 |
| 49 LEU |         | ; 0.0  |         |
| #N     |         | 55 LYS |         |
| HN     | 7.190   | N      | 123.340 |
| CA     | 53.585  | HN     | 8.790   |
| HA     | 4.342   | CA     | 60.020  |
| #CB    |         | HA     | 3.885   |
| HB#    | 1.800   | CB     | 32.390  |
| #CG    |         | HB1    | 1.770   |
| HG     | 1.800   | HB2    | 1.980   |
| CD1    | 22.340  | CG     | 24.773  |
| HD1#   | 0.628   | HG#    | 1.483   |
| CD2    | 26.025  | CD     | 28.895  |
| HD2#   | 0.727   | HD1    | 1.650   |
| C      | 176.900 | HD2    | 1.730   |
| ; 0.0  |         | CE     | 42.343  |
| 50 GLY |         | HE#    | 3.039   |
| N      | 106.865 | C      | 178.300 |
| HN     | 7.750   | ; 0.0  |         |
| CA     | 45.657  | 56 GLU |         |
| HA1    | 3.704   | N      | 118.344 |
| HA2    | 4.115   | HN     | 8.800   |
| C      | 174.600 | CA     | 60.597  |
| ; 0.0  |         | HA     | 4.082   |
| 51 GLN |         | #CB    |         |
| N      | 119.405 | HB1    | 1.940   |
| HN     | 7.953   | HB2    | 2.075   |
| CA     | 53.850  | CG     | 37.537  |
| HA     | 4.426   | HG1    | 2.310   |
| CB     | 30.593  | HG2    | 2.505   |
| HB1    | 1.646   | CD     | 184.600 |
| HB2    | 2.130   | C      | 180.100 |
| CG     | 33.612  | ; 0.0  |         |
| HG1    | 2.125   | 57 GLU |         |
| HG2    | 2.193   | N      | 122.582 |
| CD     | 180.000 | HN     | 7.771   |
| NE2    | 113.610 | CA     | 60.370  |
| HE21   | 7.000   | HA     | 4.005   |
| HE22   | 7.300   | #CB    |         |
| C      | 174.300 | #HB1   |         |
| ; 0.0  |         | #HB2   |         |
| 52 ASN |         | #CG    |         |
| N      | 118.036 | HG#    | 2.310   |
| HN     | 8.661   | CD     | 180.000 |
| CA     | 50.952  | C      | 179.500 |
| HA     | 5.136   | ; 0.0  |         |
| CB     | 39.240  | 58 LEU |         |
| HB1    | 2.530   | N      | 121.589 |
| HB2    | 2.779   | HN     | 7.957   |

|        |         |        |         |
|--------|---------|--------|---------|
| CA     | 58.000  | 64 GLU |         |
| HA     | 4.025   | N      | 116.159 |
| #CB    |         | HN     | 7.268   |
| HB#    | 2.080   | CA     | 58.850  |
| CG     | 26.650  | HA     | 4.035   |
| HG     | 1.795   | #CB    |         |
| CD1    | 23.602  | HB1    | 2.050   |
| HD1#   | 0.791   | HB2    | 2.160   |
| CD2    | 25.560  | CG     | 37.200  |
| HD2#   | 0.846   | HG1    | 2.320   |
| C      | 178.600 | HG2    | 2.510   |
| ; 0.0  |         | CD     | 184.600 |
| 59 ASP |         | C      | 179.400 |
| N      | 120.110 | ; 0.0  |         |
| HN     | 8.538   | 65 VAL |         |
| CA     | 57.250  | N      | 108.262 |
| HA     | 4.300   | HN     | 7.260   |
| CB     | 40.098  | CA     | 60.760  |
| HB1    | 2.628   | HA     | 4.595   |
| HB2    | 2.708   | CB     | 33.025  |
| CG     | 178.900 | HB     | 2.468   |
| C      | 179.000 | CG1    | 18.960  |
| ; 0.0  |         | HG1#   | 0.884   |
| 60 ALA |         | CG2    | 21.740  |
| N      | 122.952 | HG2#   | 0.850   |
| HN     | 7.630   | C      | 175.400 |
| CA     | 54.810  | HG#    | 0.860   |
| HA     | 4.179   | ; 0.0  |         |
| CB     | 17.700  | 66 ASP |         |
| HB#    | 1.503   | #N     |         |
| C      | 180.300 | #HN    |         |
| ; 0.0  |         | CA     | 53.510  |
| 61 ILE |         | HA     | 4.700   |
| N      | 121.509 | CB     | 42.300  |
| HN     | 7.574   | HB1    | 2.600   |
| CA     | 64.995  | HB2    | 2.750   |
| HA     | 3.706   | CG     | 180.000 |
| CB     | 38.213  | C      | 177.300 |
| HB     | 1.970   | ; 0.0  |         |
| CG1    | 28.924  | 67 GLU |         |
| HG11   | 1.049   | N      | 128.700 |
| HG12   | 1.891   | HN     | 8.530   |
| CD1    | 13.730  | CA     | 59.210  |
| HD1#   | 0.863   | HA     | 4.230   |
| CG2    | 17.058  | #CB    |         |
| HG2#   | 0.830   | HB1    | 2.030   |
| C      | 178.300 | HB2    | 2.250   |
| ; 0.0  |         | #CG    |         |
| 62 ILE |         | HG1    | 2.250   |
| N      | 119.304 | HG2    | 2.370   |
| HN     | 7.457   | CD     | 180.000 |
| CA     | 64.982  | C      | 176.900 |
| HA     | 3.424   | ; 0.0  |         |
| CB     | 37.588  | 68 ASP |         |
| HB     | 1.980   | N      | 116.200 |
| CG1    | 29.221  | HN     | 8.005   |
| HG11   | 1.012   | CA     | 52.375  |
| HG12   | 1.688   | HA     | 4.734   |
| CD1    | 12.516  | CB     | 40.488  |
| HD1#   | 0.768   | HB1    | 2.758   |
| CG2    | 16.278  | HB2    | 3.111   |
| HG2#   | 0.644   | CG     | 180.000 |
| C      | 177.700 | C      | 177.700 |
| ; 0.0  |         | ; 0.0  |         |
| 63 GLU |         | 69 GLY |         |
| N      | 119.108 | N      | 110.413 |
| HN     | 8.347   | HN     | 7.755   |
| CA     | 59.255  | CA     | 47.100  |
| HA     | 3.995   | HA1    | 3.805   |
| CB     | 29.673  | HA2    | 3.875   |
| HB1    | 2.055   | C      | 175.300 |
| HB2    | 2.145   | ; 0.0  |         |
| #CG    |         | 70 SER |         |
| #HG1   |         | N      | 118.246 |
| #HG2   |         | HN     | 8.543   |
| CD     | 180.000 | CA     | 60.080  |
| C      | 178.200 | HA     | 4.220   |
| ; 0.0  |         | CB     | 64.893  |

|        |         |        |         |
|--------|---------|--------|---------|
| HB1    | 3.996   | CG     | 36.270  |
| HB2    | 4.220   | HG#    | 2.330   |
| C      | 176.200 | CD     | 180.000 |
| ; 0.0  |         | C      | 180.300 |
| 71 GLY |         | ; 0.0  |         |
| N      | 118.030 | 77 GLU |         |
| HN     | 10.970  | N      | 120.723 |
| CA     | 45.555  | HN     | 8.193   |
| HA1    | 3.440   | CA     | 57.930  |
| HA2    | 4.126   | HA     | 4.168   |
| C      | 172.800 | #CB    |         |
| ; 0.0  |         | HB#    | 1.990   |
| 72 THR |         | #CG    |         |
| N      | 109.260 | HG1    | 2.340   |
| HN     | 7.685   | HG2    | 2.470   |
| CA     | 58.110  | CD     | 180.000 |
| HA     | 4.915   | C      | 178.800 |
| CB     | 73.680  | ; 0.0  |         |
| HB     | 3.674   | 78 PHE |         |
| CG2    | 22.140  | N      | 123.381 |
| HG2#   | 1.023   | HN     | 8.830   |
| C      | 173.500 | CA     | 61.167  |
| ; 0.0  |         | HA     | 4.037   |
| 73 ILE |         | CB     | 40.087  |
| N      | 126.510 | HB1    | 3.268   |
| HN     | 9.188   | HB2    | 3.430   |
| CA     | 60.427  | #CD#   |         |
| HA     | 5.069   | #HD#   | 7.100   |
| CB     | 40.585  | HD1    | 7.100   |
| HB     | 2.007   | HD2    | 7.100   |
| CG1    | 27.485  | #CE1   |         |
| HG11   | 0.883   | #HE#   | 7.270   |
| HG12   | 1.639   | HE1    | 7.280   |
| CD1    | 13.630  | HE2    | 7.280   |
| HD1#   | 0.850   | #CZ    |         |
| CG2    | 18.006  | #HZ    |         |
| HG2#   | 1.318   | C      | 177.300 |
| C      | 176.200 | ; 0.0  |         |
| ; 0.0  |         | 79 LEU |         |
| 74 ASP |         | N      | 119.767 |
| N      | 131.870 | HN     | 8.018   |
| HN     | 9.532   | CA     | 57.960  |
| CA     | 53.058  | HA     | 3.466   |
| HA     | 5.319   | #CB    |         |
| CB     | 41.458  | HB1    | 0.880   |
| HB1    | 2.753   | HB2    | 1.840   |
| HB2    | 3.382   | CG     | 25.100  |
| CG     | 178.700 | HG     | 1.100   |
| C      | 175.900 | CD1    | 25.994  |
| ; 0.0  |         | HD1#   | 0.565   |
| 75 PHE |         | CD2    | 23.507  |
| N      | 119.711 | HD2#   | 0.660   |
| HN     | 8.790   | C      | 179.100 |
| CA     | 61.483  | ; 0.0  |         |
| HA     | 3.593   | 80 VAL |         |
| CB     | 38.610  | N      | 120.323 |
| HB1    | 2.058   | HN     | 7.289   |
| HB2    | 2.500   | CA     | 57.236  |
| #CD1   |         | HA     | 3.267   |
| #HD#   | 6.680   | CB     | 31.155  |
| HD1    | 6.680   | HB     | 2.462   |
| HD2    | 6.680   | CG1    | 21.385  |
| #CE1   |         | HG1#   | 0.827   |
| #HE#   | 7.090   | CG2    | 24.047  |
| HE1    | 7.090   | HG2#   | 1.066   |
| HE2    | 7.090   | C      | 178.200 |
| #CZ    |         | ; 0.0  |         |
| #HZ    |         | 81 MET |         |
| C      | 176.400 | N      | 121.474 |
| ; 0.0  |         | HN     | 7.848   |
| 76 GLU |         | CA     | 59.255  |
| N      | 117.316 | HA     | 3.692   |
| HN     | 7.771   | CB     | 33.420  |
| CA     | 58.795  | HB1    | 1.962   |
| HA     | 3.803   | HB2    | 2.160   |
| CB     | 27.980  | CG     | 31.723  |
| HB1    | 1.905   | HG1    | 2.180   |
| HB2    | 1.995   | HG2    | 2.456   |

|        |         |        |         |
|--------|---------|--------|---------|
| CE     | 17.070  | HB2    | 2.365   |
| HE#    | 1.856   | CG     | 32.478  |
| C      | 178.200 | HG1    | 2.687   |
| ; 0.0  |         | HG2    | 2.870   |
| 82 MET |         | CE     | 17.090  |
| N      | 118.572 | HE#    | 2.277   |
| HN     | 8.070   | C      | 177.600 |
| CA     | 56.395  | ; 0.0  |         |
| HA     | 4.050   | 87 LYS |         |
| CB     | 31.718  | N      | 120.418 |
| HB1    | 0.919   | HN     | 7.856   |
| HB2    | 1.450   | CA     | 57.457  |
| CG     | 32.983  | HA     | 4.165   |
| HG1    | 1.077   | CB     | 33.062  |
| HG2    | 1.560   | HB1    | 1.663   |
| #CE    |         | HB2    | 1.783   |
| #HE#   |         | CG     | 25.132  |
| C      | 179.400 | HG1    | 1.216   |
| ; 0.0  |         | HG2    | 1.343   |
| 83 VAL |         | #CD    |         |
| N      | 122.284 | #HD1   |         |
| HN     | 8.377   | #HD2   |         |
| CA     | 66.887  | #CE    |         |
| HA     | 3.643   | #HE1   |         |
| CB     | 31.518  | #HE2   |         |
| HB     | 2.041   | C      | 177.100 |
| CG1    | 21.324  | ; 0.0  |         |
| HG1#   | 0.651   | 88 GLU |         |
| CG2    | 24.248  | N      | 121.903 |
| HG2#   | 1.203   | HN     | 7.907   |
| C      | 178.100 | CA     | 56.960  |
| ; 0.0  |         | HA     | 4.300   |
| 84 ARG |         | CB     | 29.200  |
| N      | 120.848 | HB1    | 2.005   |
| HN     | 8.060   | HB2    | 2.080   |
| CA     | 59.360  | CG     | 36.600  |
| HA     | 3.860   | HG1    | 2.280   |
| CB     | 30.120  | HG2    | 2.340   |
| HB#    | 1.865   | CD     | 180.000 |
| CG     | 27.460  | C      | 176.500 |
| HG1    | 1.586   | ; 0.0  |         |
| HG2    | 1.790   | 89 ASP |         |
| CD     | 43.698  | N      | 122.306 |
| HD#    | 3.074   | HN     | 8.079   |
| NE     | 85.700  | CA     | 54.035  |
| HE     | 7.190   | HA     | 4.630   |
| #NH1   |         | CB     | 41.267  |
| #HH11  |         | HB1    | 2.598   |
| #HH12  |         | HB2    | 2.740   |
| #NH2   |         | CG     | 180.600 |
| #HH21  |         | C      | 174.900 |
| #HH22  |         | ; 0.0  |         |
| C      | 179.000 | 90 ALA |         |
| ; 0.0  |         | N      | 130.700 |
| 85 GLN |         | HN     | 7.646   |
| N      | 117.195 | CA     | 53.730  |
| HN     | 7.400   | HA     | 4.117   |
| CA     | 57.330  | CB     | 20.270  |
| HA     | 4.153   | HB#    | 1.345   |
| #CB    |         | C      | 170.600 |
| #HB1   |         | ; 0.0  |         |
| HB2    | 2.140   |        |         |
| CG     | 33.500  |        |         |
| HG1    | 2.390   |        |         |
| HG2    | 2.470   |        |         |
| CD     | 179.500 |        |         |
| NE2    | 111.890 |        |         |
| HE21   | 6.725   |        |         |
| HE22   | 7.370   |        |         |
| C      | 177.900 |        |         |
| ; 0.0  |         |        |         |
| 86 MET |         |        |         |
| N      | 120.148 |        |         |
| HN     | 7.924   |        |         |
| CA     | 57.918  |        |         |
| HA     | 4.247   |        |         |
| CB     | 33.944  |        |         |
| HB1    | 2.197   |        |         |



APPENDIX **C****NOE AND STRUCTURE  
CALCULATION PARAMETERS****1. NOE calibration**

This is a "nawk" program that take as input a file with the following format:

```
THR   4 HN   THR   4 HA   3380
ASP-  5 HN   ASP-  5 HA  16000
GLN   6 HN   ASP-  5 HA   7010
```

nawk program :

```
grep -v "GLY" $1 \
|nawk '{
diff=$2-$5
if (diff==0) {
    dna[$2]=1
    dna_N[$2]="1:$1_"$2":$3
    dna_A[$2]="1:$4_"$5":$6
    dna_I[$2]=$7
}
if (diff==1) {
    dan[$2]=1
    dan_N[$2]="1:$1_"$2":$3
    dan_A[$2]="1:$4_"$5":$6
    dan_I[$2]=$7
}
if (diff==3) {
    dan3[$2]=1
    dan3_N[$2]="1:$1_"$2":$3
    dan3_A[$2]="1:$4_"$5":$6
    dan3_I[$2]=$7
}
}
END{
#####
#           CASE           Dna_rstrnt           Dan_rstrnt
#           =====           =====           =====
#           no Dna/Dan       (L0, U0)           (L1, U1)
#           Dna only         (L0, U0)           (L1, U1)
#           Dan only         (L0, U0)           (L1, U1)
#           Dna >> Dan       (2.6, U0)          (U0, U1)
```

```

#          Dna > Dan          (2.6, U0)          (2.9, U1)
#          Dna << Dan         (2.7, U0)          (L1, 2.7)
#          Dna < Dan          (2.7, U0)          (L1, 2.9)
#          Dna ~ Dan          (2.6, U0)          (2.3, U1)
#
#####
# note :   >>      :      ratio > 1.5
#           >      :      1.5 > ratio > 1.2
#           ~      :      0.833 < ratio < 1.2

for (i=1; i<=90; i++) {
  if ((dna[i]==1)&&(dan[i]==1)){
    pr_dna(dna_N[i],dna_A[i],dan_A[i],dna_I[i],dan_I[i])
  }
  if ((dna[i]==1)&&(dan[i]==0)) pr(dna_N[i],dna_A[i],1.8,5.0,dna_I[i])
  if ((dna[i]==0)&&(dan[i]==1)) pr(dan_N[i],dan_A[i],1.8,5.0,dan_I[i])

  if (dan3[i]==1) {
    if ((dna[i]==1)&&(dan[i]==1)) {
      prdan3_11(dan3_N[i],dan3_A[i],dna_I[i],dan_I[i],dan3_I[i])
    }
    if ((dna[i]==1)&&(dan[i]==0)) {
      prdan3_10(dan3_N[i],dan3_A[i],dna_I[i],dan3_I[i])
    }
    if ((dna[i]==0)&&(dan[i]==1)) {
      prdan3_01(dan3_N[i],dan3_A[i],dan_I[i],dan3_I[i])
    }
    if ((dna[i]==0)&&(dan[i]==0)) {
      pr(dan3_N[i],dan3_A[i],1.8,5.0,dan3_I[i])
    }
  }
}

func prdan3_11 (HN,HA,dna,dan,dan3) {
  if ((dan3/dna) > 1.2) {pr(HN,HA,1.8,3.1,dan3) ; return}
  if ((dna/dan3) > 1.2) {
    if ((dan/dan3) > 1.2) {pr(HN,HA,2.8,5.0,dan3) ; return}
    if ((dan3/dan) > 1.2) {pr(HN,HA,2.8,3.6,dan3) ; return}
    pr(HN,HA,2.8,4.3,dan3)
    return
  }
  if ((dan/dan3) > 1.2) {pr(HN,HA,2.2,4.1,dan3) ; return}
  if ((dan3/dan) > 1.2) {pr(HN,HA,2.2,3.6,dan3) ; return}
  pr(HN,HA,2.2,4.3,dan3)
  return
}

function prdan3_10(HN,HA,dna,dan3) {
  if ((dan3/dna) > 1.2) {pr(HN,HA,1.8,3.1,dan3) ; return}
  if ((dna/dan3) > 1.2) {pr(HN,HA,2.8,5.0,dan3) ; return}
  pr(HN,HA,2.8,4.1,dan3)
  return
}

function prdan3_01(HN,HA,dan,dan3) {
  if ((dan3/dan) > 1.2) {pr(HN,HA,1.8,3.6,dan3) ; return}
  if ((dan/dan3) > 1.2) {pr(HN,HA,2.8,5.0,dan3) ; return}
  pr(HN,HA,1.9,4.3,dan3)
  return
}

```

```

    }

function pr_dna(HN,HA0,HA1,dna,dan){
    if (dna > dan) {
        if ((dna/dan)>1.5) {
            pr(HN,HA0,2.6,3.1,dna)
            pr(HN,HA1,3.1,3.6,dan)
            return
        }
        if ((dna/dan)>1.2) {
            pr(HN,HA0,2.6,3.1,dna)
            pr(HN,HA1,2.9,3.6,dan)
            return
        }
    }
    if (dan > dna) {
        if ((dan/dna)>1.5) {
            pr(HN,HA0,2.7,3.1,dna)
            pr(HN,HA1,2.1,2.7,dan)
            return
        }
        if ((dan/dna)>1.2) {
            pr(HN,HA0,2.7,3.1,dna)
            pr(HN,HA1,2.1,2.9,dan)
            return
        }
    }
    pr(HN,HA0,2.6,3.1,dna)
    pr(HN,HA1,2.3,3.6,dan)
    return
}

function pr(HN,HA,LO,UP,NOE) {
    printf "%-15s%-14s%6.1f%6.1f%8.1f   10 10 1000\n",HN,HA,LO,UP,UP,NOE
    return
}'
grep "GLY" $1 \
|nawk '{
dna_N="1:"$1_"$2": "$3
dna_A="1:"$4_"$5": "$6
printf "%-15s%-15s   1.8       5.0           5.0   10 10 1000\n",dna_N,dna_A,$7}'

```

## NOE restraints

This is the Biosym DGII restraint file used to calculate the preliminary structure of NTnC-2Ca :

```
!BIOSYM restraint 1
!
#remote_prochiral_center
1:VAL_83:HG2* 1:VAL_83:HG1* 1:VAL_83:CG2 1:VAL_83:CG1 1:VAL_83:CB
1:LEU_79:HD2* 1:LEU_79:HD1* 1:LEU_79:CD2 1:LEU_79:CD1 1:LEU_79:CG
1:VAL_65:HG1* 1:VAL_65:HG2* 1:VAL_65:CG1 1:VAL_65:CG2 1:VAL_65:CB
1:VAL_80:HG2* 1:VAL_80:HG1* 1:VAL_80:CG2 1:VAL_80:CG1 1:VAL_80:CB
1:VAL_45:HG2* 1:VAL_45:HG1* 1:VAL_45:CG2 1:VAL_45:CG1 1:VAL_45:CB
1:LEU_14:HD1* 1:LEU_14:HD2* 1:LEU_14:CD1 1:LEU_14:CD2 1:LEU_14:CG
1:LEU_58:HD1* 1:LEU_58:HD2* 1:LEU_58:CD1 1:LEU_58:CD2 1:LEU_58:CG
1:LEU_42:HD1* 1:LEU_42:HD2* 1:LEU_42:CD1 1:LEU_42:CD2 1:LEU_42:CG
1:LEU_49:HD2* 1:LEU_49:HD1* 1:LEU_49:CD2 1:LEU_49:CD1 1:LEU_49:CG
!
#chiral
1:ALAN_1:CA S
1:SER_2:CA S
1:MET_3:CA S
1:THR_4:CA S
1:THR_4:CB R
1:ASP-_5:CA S
1:GLN_6:CA S
1:GLN_7:CA S
1:ALA_8:CA S
1:GLU-_9:CA S
1:ALA_10:CA S
1:ARG+_11:CA S
1:ALA_12:CA S
1:PHE_13:CA S
1:LEU_14:CA S
1:SER_15:CA S
1:GLU-_16:CA S
1:GLU-_17:CA S
1:MET_18:CA S
1:ILE_19:CA S
1:ILE_19:CB S
1:ALA_20:CA S
1:GLU-_21:CA S
1:PHE_22:CA S
1:LYS+_23:CA S
1:ALA_24:CA S
1:ALA_25:CA S
1:PHE_26:CA S
1:ASP-_27:CA S
1:MET_28:CA S
1:PHE_29:CA S
1:ASP-_30:CA S
1:ALA_31:CA S
1:ASP-_32:CA S
1:ASP-_36:CA S
1:ILE_37:CA S
1:ILE_37:CB S
1:SER_38:CA S
1:THR_39:CA S
1:THR_39:CB R
1:LYS+_40:CA S
1:GLU-_41:CA S
1:LEU_42:CA S
1:THR_44:CA S
1:THR_44:CB R
1:VAL_45:CA S
1:MET_46:CA S
1:ARG+_47:CA S
1:MET_48:CA S
1:LEU_49:CA S
1:GLN_51:CA S
1:ASN_52:CA S
```

```

1:PRO_53:CA      S
1:THR_54:CA      S
1:THR_54:CB      R
1:LYS+_55:CA     S
1:GLU-_56:CA     S
1:GLU-_57:CA     S
1:LEU_58:CA      S
1:ASP-_59:CA     S
1:ALA_60:CA      S
1:ILE_61:CA      S
1:ILE_61:CB      S
1:ILE_62:CA      S
1:ILE_62:CB      S
1:GLU-_63:CA     S
1:GLU-_64:CA     S
1:VAL_65:CA      S
1:ASP-_66:CA     S
1:GLU-_67:CA     S
1:ASP-_68:CA     S
1:SER_70:CA      S
1:THR_72:CA      S
1:THR_72:CB      R
1:ILE_73:CA      S
1:ILE_73:CB      S
1:ASP-_74:CA     S
1:PHE_75:CA      S
1:GLU-_76:CA     S
1:GLU-_77:CA     S
1:PHE_78:CA      S
1:LEU_79:CA      S
1:VAL_80:CA      S
1:MET_81:CA      S
1:MET_82:CA      S
1:VAL_83:CA      S
1:ARG+_84:CA     S
1:GLN_85:CA      S
1:MET_86:CA      S
1:LYS+_87:CA     S
1:GLU-_88:CA     S
1:ASP-_89:CA     S
1:ALAC_90:CA    S
!
#NOE_distance
1:ILE_62:HD1*    1:ILE_62:HG11    1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:ILE_62:HD1*    1:THR_39:HG2*    1.800  8.000  5.000 10.00 10.00 1000.000 0.00
1:ILE_62:HD1*    1:ILE_62:HA      1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:ILE_62:HD1*    1:THR_39:HA      1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:ILE_62:HD1*    1:THR_39:HB      1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:ILE_62:HD1*    1:ASP-_59:HA     1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:ILE_62:HD1*    1:THR_72:HA      1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:LYS+_55:HE*     1:LYS+_55:HA      1.800  6.000  5.000 10.00 10.00 1000.000 0.00
1:LYS+_23:HE*     1:LYS+_23:HG*     1.800  7.000  5.000 10.00 10.00 1000.000 0.00
1:LYS+_23:HE*     1:LYS+_23:HB*     1.800  7.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_89:HB2     1:ASP-_89:HB1     1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_89:HB2     1:ASP-_89:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_74:HB2     1:ASP-_74:HB1     1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_74:HB2     1:ASP-_74:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_74:HB1     1:ASP-_74:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_36:HB2     1:ASP-_36:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_36:HB1     1:ASP-_36:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_36:HB2     1:ASP-_36:HB1     1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_89:HB1     1:ASP-_89:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:PHE_13:HB1     1:VAL_83:HG2*     1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:PHE_13:HB1     1:PHE_13:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:PHE_13:HB2     1:PHE_13:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ILE_73:HB       1:ILE_73:HG2*     1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:ILE_73:HB       1:ILE_73:HG12     1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_68:HB2     1:ASP-_68:HB1     1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_68:HB1     1:ASP-_68:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_68:HB2     1:ASP-_68:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:PHE_78:HB2     1:ILE_73:HG2*     1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:PHE_78:HB1     1:ILE_73:HG2*     1.800  6.500  5.000 10.00 10.00 1000.000 0.00
1:PHE_78:HB2     1:PHE_78:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:PHE_78:HB1     1:PHE_78:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:PHE_78:HB2     1:PHE_78:HB1     1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_59:HB1     1:GLU-_56:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_59:HB2     1:GLU-_56:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00
1:ASP-_59:HB2     1:ASP-_59:HA      1.800  5.000  5.000 10.00 10.00 1000.000 0.00

```

|              |               |       |       |       |       |       |          |      |
|--------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:ASP_59:HB1 | 1:ASP_59:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HB  | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HB  | 1:ILE_37:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HB  | 1:ILE_73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HB  | 1:ILE_73:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_32:HB2 | 1:ASP_32:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_32:HB2 | 1:ASP_32:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_32:HB1 | 1:ASP_32:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_27:HB2 | 1:ASP_27:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_27:HB1 | 1:ASP_27:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_5:HB2  | 1:ASP_5:HA    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HB2 | 1:ASN_52:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HB1 | 1:PRO_53:HD2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HB2 | 1:ASN_52:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HB1 | 1:ASN_52:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HB2 | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HB1 | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HB1 | 1:PHE_75:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HB2 | 1:PHE_29:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HB1 | 1:PHE_29:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_30:HB1 | 1:ASP_30:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_30:HB2 | 1:ASP_30:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_30:HB1 | 1:ASP_30:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_30:HB1 | 1:ASP_27:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_30:HB2 | 1:ASP_27:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HB  | 1:ILE_62:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HB  | 1:ILE_61:HG11 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HB  | 1:ILE_61:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HB  | 1:ILE_62:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HB  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HB  | 1:ASP_59:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HB* | 1:LEU_79:HD2* | 1.800 | 7.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HB* | 1:ILE_19:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HB* | 1:PHE_22:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HB  | 1:ILE_19:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HB  | 1:ILE_19:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HB  | 1:ILE_19:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HB  | 1:ILE_19:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HB  | 1:GLU_16:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HB  | 1:ILE_62:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HB  | 1:ILE_62:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_56:HG2 | 1:GLU_56:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_56:HG2 | 1:GLU_56:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_76:HG* | 1:GLU_76:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HG2  | 1:GLN_6:HB2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HG1  | 1:GLN_6:HB2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HG2  | 1:GLN_6:HG1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HB1 | 1:MET_86:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HB2 | 1:MET_86:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HB1 | 1:MET_86:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HB2 | 1:VAL_83:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HB1 | 1:VAL_83:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HB1 | 1:MET_86:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HG2 | 1:MET_46:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HG1 | 1:MET_46:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HG2 | 1:MET_46:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HG1 | 1:MET_46:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HG1 | 1:MET_46:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HB1 | 1:MET_81:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HG2 | 1:GLN_51:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HG1 | 1:GLN_51:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HG2 | 1:GLY_50:HA1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HG2 | 1:GLN_51:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HG1 | 1:GLN_51:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS_87:HB2 | 1:LYS_87:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS_87:HB1 | 1:LYS_87:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HG1 | 1:MET_82:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HG2 | 1:MET_82:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HG1 | 1:MET_82:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HG2 | 1:MET_82:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HB  | 1:VAL_65:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HB  | 1:VAL_65:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HG1 | 1:MET_48:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HG2 | 1:MET_48:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HG1 | 1:MET_48:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HG2 | 1:MET_48:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HG2 | 1:MET_48:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HG1 | 1:MET_48:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |



|               |               |       |       |       |       |       |          |      |
|---------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:MET_28:HG1  | 1:MET_28:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HB2  | 1:MET_18:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HB2  | 1:MET_18:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HB1  | 1:MET_18:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HB1  | 1:MET_18:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HB2  | 1:MET_18:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HG2  | 1:MET_86:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HG2  | 1:MET_86:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HB2 | 1:LYS+_55:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HB2 | 1:LYS+_55:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HB1 | 1:LYS+_55:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HB1  | 1:MET_48:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HB1  | 1:MET_48:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HB2  | 1:MET_48:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HB2  | 1:MET_48:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HB1  | 1:MET_48:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HB2 | 1:LYS+_40:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HB2 | 1:LYS+_40:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HB1 | 1:LYS+_40:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HG2  | 1:MET_18:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HG2  | 1:MET_18:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HG2  | 1:MET_18:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HG1  | 1:MET_18:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HB2  | 1:MET_28:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HB2  | 1:MET_28:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HB1  | 1:MET_28:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HB2  | 1:PRO_53:HD1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HB2  | 1:PRO_53:HD2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HB1  | 1:PRO_53:HD2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HB2  | 1:PRO_53:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HB1  | 1:PRO_53:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HB1  | 1:MET_46:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HB2  | 1:MET_46:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HB2  | 1:MET_46:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HB1  | 1:MET_46:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HB1  | 1:MET_46:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HB   | 1:VAL_83:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HB   | 1:VAL_83:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HB   | 1:ALA_10:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HB2  | 1:MET_82:HB*  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HB2  | 1:MET_82:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HG1  | 1:MET_81:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HG1  | 1:MET_81:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HG2  | 1:MET_81:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HG2  | 1:MET_81:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HG1  | 1:MET_81:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HG2  | 1:MET_81:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HB   | 1:VAL_80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HB   | 1:VAL_80:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_45:HB   | 1:VAL_45:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_45:HB   | 1:VAL_45:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HB* | 1:LYS+_23:HA  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HB* | 1:ILE_19:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HB2  | 1:GLN_51:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HB2  | 1:GLY_50:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HB2  | 1:GLN_51:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HB1  | 1:GLN_51:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HB1   | 1:GLN_6:HA    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HB2   | 1:GLN_6:HA    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HB* | 1:ARG+_84:HD* | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HB* | 1:ARG+_47:HD* | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HB* | 1:ARG+_47:HA  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_63:HB1 | 1:ALA_60:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_63:HB2 | 1:ALA_60:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_63:HB1 | 1:GLU-_63:HB* | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_63:HB2 | 1:ALA_60:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_63:HB1 | 1:ALA_60:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_63:HB2 | 1:GLU-_63:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG12 | 1:ILE_62:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG12 | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG11 | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG11 | 1:ILE_62:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG11 | 1:ILE_62:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG12 | 1:ILE_62:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HD* | 1:LYS+_40:HE* | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HD* | 1:LYS+_40:HA  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HG11 | 1:ILE_61:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HG12 | 1:ILE_61:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |



|               |               |       |       |       |       |       |          |      |
|---------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:ILE_61:HG11 | 1:ILE_61:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HG12 | 1:ILE_61:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HD1 | 1:LYS+_55:HE* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HD2 | 1:LYS+_55:HE* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_17:HB1 | 1:GLU-_17:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HG1 | 1:ARG+_47:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HG2 | 1:ARG+_47:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HG1 | 1:ARG+_47:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HG2 | 1:ARG+_47:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HD1* | 1:LEU_14:HD2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HD1* | 1:ALA_10:HB*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HD1* | 1:ARG+_11:HA  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HD1* | 1:LEU_14:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_76:HB1 | 1:GLU-_76:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_76:HB2 | 1:GLU-_76:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HG11 | 1:ILE_73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HG2 | 1:ARG+_84:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HG2 | 1:ARG+_84:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HG2 | 1:ARG+_84:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HG1 | 1:ARG+_84:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HG11 | 1:ILE_73:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HG12 | 1:ILE_73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HG11 | 1:ILE_73:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HD2 | 1:LYS+_23:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HD1 | 1:LYS+_23:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HD1 | 1:LYS+_23:HE* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HD2 | 1:LYS+_23:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HG   | 1:PRO_53:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HG   | 1:LEU_58:HB*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HG1* | 1:ILE_37:HD1* | 1.800 | 7.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HG1* | 1:ILE_37:HG2* | 1.800 | 7.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HG1* | 1:ILE_37:HB   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HG1* | 1:ILE_37:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HD1 | 1:LYS+_23:HD2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HD2 | 1:LYS+_23:HE* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HG   | 1:LEU_58:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HG   | 1:LEU_58:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HG   | 1:LYS+_55:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HG   | 1:LEU_58:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HG   | 1:LEU_42:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HG   | 1:LEU_42:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HG   | 1:LEU_14:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HG   | 1:LEU_14:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HD1* | 1:LEU_79:HG   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HD1* | 1:ALA_10:HB*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HD1* | 1:LEU_79:HB2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HD1* | 1:LEU_79:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_49:HD2* | 1:MET_46:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_49:HD2* | 1:MET_46:HG2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_49:HD2* | 1:MET_46:HG1  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HD2* | 1:LEU_58:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HD1* | 1:LEU_42:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HG2 | 1:LYS+_87:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HG1 | 1:LYS+_87:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HG1 | 1:LYS+_87:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HG1 | 1:LYS+_87:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HG2 | 1:LYS+_87:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HG* | 1:LYS+_55:HE* | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HG* | 1:LYS+_55:HA  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HG* | 1:LYS+_55:HD1 | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HG1 | 1:LYS+_40:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HG1 | 1:LYS+_40:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HG2 | 1:LYS+_40:HE* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HG1 | 1:LYS+_40:HE* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HG2 | 1:LYS+_40:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HG1 | 1:LYS+_40:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HD2* | 1:LEU_14:HB2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HD2* | 1:MET_86:HE*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HG2* | 1:VAL_83:HG1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HG2* | 1:VAL_83:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HG2* | 1:VAL_80:HG1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HG2* | 1:GLU-_77:HA  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HD2* | 1:LEU_42:HB2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HD2* | 1:LEU_42:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HG2* | 1:ALA_10:HB*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HG2* | 1:ALA_10:HB*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HD2* | 1:LEU_79:HG   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HD2* | 1:LEU_79:HB2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |

|               |               |       |       |       |       |       |          |      |
|---------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:LEU_58:HD1* | 1:PRO_53:HB2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HD1* | 1:LEU_58:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HD1* | 1:GLY_43:HA1  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HG2* | 1:ILE_62:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HG2* | 1:ASP_59:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HG2* | 1:GLY_71:HA2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_49:HD1* | 1:LEU_49:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HG2* | 1:VAL_65:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HG2* | 1:LEU_58:HD1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HG2* | 1:MET_48:HE*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HG1* | 1:ARG_84:HD*  | 1.800 | 7.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALAC_90:HB* | 1:ALAC_90:HA  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HG1* | 1:ILE_73:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HG2* | 1:VAL_65:HB   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_25:HB*  | 1:MET_48:HE*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_25:HB*  | 1:ALA_24:HB*  | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_25:HB*  | 1:PHE_22:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HB*  | 1:VAL_83:HG1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HB*  | 1:GLN_7:HA    | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HE*  | 1:VAL_45:HG1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HE*  | 1:VAL_45:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HE*  | 1:VAL_45:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HE*  | 1:MET_48:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HE*  | 1:MET_46:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HE*  | 1:MET_28:HG1  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HE*  | 1:MET_28:HG2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HE*  | 1:MET_28:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HG2* | 1:LEU_79:HD1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HG2* | 1:PHE_22:HB*  | 1.800 | 7.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HE*  | 1:LEU_14:HD2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HE*  | 1:MET_86:HG1  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HE*  | 1:MET_86:HG2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HE*  | 1:VAL_83:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HE*  | 1:MET_86:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HE*  | 1:ILE_73:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HE*  | 1:MET_81:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HG2* | 1:ILE_61:HG11 | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HG2* | 1:ILE_61:HG12 | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HG2* | 1:ILE_61:HB   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HG2* | 1:ILE_61:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_3:HE*   | 1:ASP_68:HB2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG2* | 1:GLU_63:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG2* | 1:ILE_62:HD1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG2* | 1:ASP_66:HB2  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HG2* | 1:THR_72:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HB1  | 1:LEU_14:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_35:HA2  | 1:GLY_35:HA1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HD1* | 1:ILE_73:HD1* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HD1* | 1:ILE_37:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HD1* | 1:ILE_73:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HD1* | 1:ILE_73:HB   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HD1* | 1:ILE_37:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HD1* | 1:ILE_73:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HD1* | 1:ILE_73:HG12 | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HD1* | 1:ILE_73:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HD1* | 1:ILE_61:HG11 | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HD1* | 1:ILE_61:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG_47:HD*  | 1:ARG_47:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HD1* | 1:ILE_19:HG2* | 1.800 | 8.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HD1* | 1:ILE_19:HA   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HB1  | 1:LEU_42:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HB2  | 1:LEU_42:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HB1  | 1:LEU_42:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HB2  | 1:THR_39:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HB1  | 1:LEU_42:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HB2  | 1:LEU_42:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_54:HB   | 1:THR_54:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_54:HB   | 1:THR_54:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_4:HB    | 1:THR_4:HA    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_4:HB    | 1:THR_4:HG2*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HB   | 1:VAL_45:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HB   | 1:THR_44:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HB   | 1:GLU_41:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HB   | 1:THR_44:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HB   | 1:LEU_42:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HB   | 1:THR_39:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HA   | 1:VAL_80:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HA   | 1:VAL_80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |

|              |               |       |       |       |       |       |          |      |
|--------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:VAL_80:HA  | 1:VAL_83:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HA  | 1:VAL_80:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HA  | 1:THR_44:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HA  | 1:ARG+_47:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HA  | 1:VAL_45:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HA  | 1:ALA_10:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HA  | 1:VAL_83:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_83:HA  | 1:VAL_83:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HA  | 1:THR_39:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HA  | 1:LEU_42:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HA  | 1:THR_39:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HB1 | 1:THR_72:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HB2 | 1:THR_72:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HA  | 1:LEU_79:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HA  | 1:ILE_19:HG11 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HA  | 1:ILE_19:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HA  | 1:MET_18:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_45:HA  | 1:VAL_45:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HB1 | 1:SER_38:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HB2 | 1:SER_38:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HB2 | 1:SER_38:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HB1 | 1:SER_15:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HB1 | 1:THR_72:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HB2 | 1:SER_70:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HA  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HA  | 1:GLU-_63:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HA  | 1:ILE_62:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HA  | 1:ALA_60:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_26:HA  | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_26:HA  | 1:ALA_25:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_26:HA  | 1:PHE_26:HB*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_26:HA  | 1:PHE_29:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HA  | 1:PHE_78:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HA  | 1:PHE_78:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HA  | 1:PHE_75:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HA  | 1:PHE_75:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_78:HA  | 1:ILE_73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_4:HA   | 1:THR_4:HG2*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HA  | 1:ILE_73:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HA  | 1:VAL_65:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_56:HA | 1:GLU-_56:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_56:HA | 1:GLU-_56:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HA  | 1:ILE_73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HA  | 1:ILE_73:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HA  | 1:SER_70:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_54:HA  | 1:THR_54:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HA  | 1:ILE_37:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HA  | 1:ILE_37:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_17:HA | 1:GLU-_17:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_17:HA | 1:GLU-_17:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_17:HA | 1:GLU-_17:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_16:HA | 1:ILE_19:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_16:HA | 1:ILE_19:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_16:HA | 1:GLU-_16:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_16:HA | 1:GLU-_16:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_16:HA | 1:GLU-_16:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_16:HA | 1:GLU-_16:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HA | 1:THR_54:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HA | 1:ARG+_84:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_81:HA  | 1:ARG+_84:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_67:HA | 1:ASP-_68:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_63:HA | 1:ASP-_66:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HA  | 1:MET_46:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HA  | 1:ARG+_47:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HA | 1:THR_39:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_21:HA | 1:PHE_22:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HA  | 1:LEU_14:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_76:HA | 1:LEU_79:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-_41:HA | 1:GLU-_41:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HA  | 1:LEU_79:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HA | 1:LYS+_23:HD1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HA  | 1:ILE_73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HA  | 1:MET_28:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HA  | 1:ILE_19:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HA   | 1:GLN_7:HG*   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HA   | 1:VAL_80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_2:HA   | 1:SER_2:HB*   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HA  | 1:MET_86:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |

|               |               |       |       |       |       |       |          |      |
|---------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:MET_86:HA   | 1:MET_86:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HA   | 1:LEU_79:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HA   | 1:LEU_79:HG   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HA   | 1:LEU_79:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HA   | 1:THR_72:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HA   | 1:THR_72:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HA   | 1:MET_28:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HA  | 1:LYS+_87:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HA  | 1:LYS+_87:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HA   | 1:ARG+_84:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-5:HA   | 1:ALA_8:HB*   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-5:HA   | 1:ASP_-5:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-59:HA  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HA   | 1:MET_82:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HA   | 1:MET_82:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HA   | 1:MET_82:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HA   | 1:SER_15:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HA   | 1:SER_15:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HA   | 1:THR_72:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HA   | 1:THR_72:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HA   | 1:VAL_83:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HA   | 1:VAL_83:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HA   | 1:ALA_10:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_31:HA   | 1:ALA_31:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_31:HA   | 1:ASP_-32:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_25:HA   | 1:ALA_25:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALAC_90:HA  | 1:ASP_-89:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_49:HA   | 1:LEU_49:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HA   | 1:LEU_14:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HA   | 1:LEU_14:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HA   | 1:ALA_12:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-74:HA  | 1:ASP_-36:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-74:HA  | 1:ASP_-36:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-36:HA  | 1:ASP_-74:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-36:HA  | 1:ASP_-74:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HA  | 1:ALA_31:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HA  | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALAN_1:HA   | 1:ALAN_1:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HA   | 1:PRO_53:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HD1  | 1:PRO_53:HG*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HD2  | 1:PRO_53:HG*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HD1  | 1:PRO_53:HD2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HD2  | 1:PRO_53:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HD1  | 1:PRO_53:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PRO_53:HD2  | 1:ASN_52:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_43:HA2  | 1:LEU_58:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_43:HA2  | 1:GLY_43:HA1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_43:HA1  | 1:LEU_58:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_71:HA2  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_71:HA1  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_71:HA2  | 1:GLY_71:HA1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_50:HA2  | 1:GLY_50:HA1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HB1  | 1:LEU_14:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HB1  | 1:LEU_14:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HB2  | 1:LEU_14:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HB2  | 1:LEU_14:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HB1  | 1:LEU_14:HG   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HD* | 1:ARG+_84:HG1 | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HD* | 1:MET_81:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HB   | 1:ASP_-36:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HB   | 1:ASP_-36:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HB   | 1:GLY_71:HA2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HB   | 1:ASP_-36:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HB   | 1:THR_72:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HD* | 1:ASN_52:HA   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HN   | 1:THR_72:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HN   | 1:ILE_73:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HN   | 1:ILE_73:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HN   | 1:THR_72:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HN   | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HN   | 1:ILE_37:HG1* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HN   | 1:ILE_37:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HN   | 1:ASP_-36:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HN  | 1:LYS+_23:HD1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HN  | 1:LYS+_23:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HN  | 1:LYS+_23:HD2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HN  | 1:LYS+_23:HB* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |



|             |              |       |       |       |       |       |          |      |
|-------------|--------------|-------|-------|-------|-------|-------|----------|------|
| 1:LYS+23:HN | 1:PHE22:HB*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+23:HN | 1:PHE22:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA10:HN  | 1:VAL80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA10:HN  | 1:ALA10:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA10:HN  | 1:GLU9:HN    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA8:HN   | 1:VAL80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA8:HN   | 1:ALA8:HB*   | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA8:HN   | 1:GLN7:HB1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA8:HN   | 1:GLN7:HB2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA8:HN   | 1:ASP5:HB1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA8:HN   | 1:GLN7:HN    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA8:HN   | 1:GLU9:HN    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER38:HN  | 1:SER38:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER38:HN  | 1:ILE37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER38:HN  | 1:ILE37:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER38:HN  | 1:GLU41:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER38:HN  | 1:LEU42:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE78:HN  | 1:ILE73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE78:HN  | 1:PHE78:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE78:HN  | 1:PHE78:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE78:HN  | 1:PHE78:HE*  | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE78:HN  | 1:LEU79:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE78:HN  | 1:GLU77:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+55:HN | 1:THR54:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+55:HN | 1:LYS+55:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+55:HN | 1:LYS+55:HD1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+55:HN | 1:LYS+55:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+55:HN | 1:LYS+55:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:ALA60:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:ASP59:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:ASP59:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:ILE61:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:ILE62:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:LEU58:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:GLU63:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA60:HN  | 1:ASP59:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:THR44:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:MET48:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:MET48:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN7:HN   | 1:VAL80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN7:HN   | 1:GLN7:HG*   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN7:HN   | 1:GLN6:HN    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP5:HN   | 1:THR4:HG2*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP5:HN   | 1:ASP5:HB1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP5:HN   | 1:ASP5:HB2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP5:HN   | 1:GLN6:HN    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU57:HN  | 1:GLU57:HG*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU57:HN  | 1:GLU56:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU57:HN  | 1:LYS+55:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU57:HN  | 1:ASP59:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU57:HN  | 1:THR54:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU57:HN  | 1:GLU56:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU57:HN  | 1:LEU58:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:LEU49:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:MET48:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:MET48:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:MET48:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:LEU49:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET48:HN  | 1:ARG47:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+40:HN | 1:LYS+40:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+40:HN | 1:LYS+40:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+40:HN | 1:LYS+40:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+40:HN | 1:THR39:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:ILE19:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:GLU16:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:GLU16:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:GLU16:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:GLU16:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:SER15:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:SER15:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU16:HN  | 1:GLU17:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA20:HN  | 1:GLU16:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP89:HN  | 1:ALAC90:HB* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP89:HN  | 1:GLU88:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP89:HN  | 1:GLU88:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP89:HN  | 1:ASP89:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP89:HN  | 1:ASP89:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP89:HN  | 1:ALAC90:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |

|             |               |       |       |       |       |       |          |      |
|-------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:ASP-89:HN | 1:GLU-88:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-83:HN | 1:MET-82:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-83:HN | 1:MET-82:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-83:HN | 1:MET-82:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-83:HN | 1:MET-82:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-83:HN | 1:VAL-83:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-83:HN | 1:VAL-83:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-83:HN | 1:VAL-83:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-42:HN | 1:ILE-37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-42:HN | 1:LEU-42:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-42:HN | 1:ILE-37:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-42:HN | 1:LEU-42:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-42:HN | 1:LEU-42:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-42:HN | 1:GLU-41:HG*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-42:HN | 1:GLU-41:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-41:HN | 1:ILE-37:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-41:HN | 1:LYS-40:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-41:HN | 1:LYS-40:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-41:HN | 1:LYS-40:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-41:HN | 1:GLU-41:HG*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-24:HN | 1:LYS-23:HG*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-24:HN | 1:LYS-23:HB*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-24:HN | 1:ALA-24:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-25:HN | 1:ALA-25:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-24:HN | 1:LYS-23:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-21:HN | 1:ILE-19:HG11 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-21:HN | 1:GLU-21:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-21:HN | 1:GLU-21:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-21:HN | 1:GLU-21:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-21:HN | 1:LYS-23:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-21:HN | 1:PHE-22:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-20:HN | 1:ILE-19:HG11 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-20:HN | 1:ILE-19:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-20:HN | 1:ALA-20:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-20:HN | 1:ILE-19:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-20:HN | 1:GLU-21:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-20:HN | 1:ILE-19:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-88:HN | 1:LYS-87:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-88:HN | 1:LYS-87:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-88:HN | 1:GLU-88:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-88:HN | 1:GLU-88:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-88:HN | 1:GLU-88:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU-88:HN | 1:GLU-88:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-45:HN | 1:VAL-45:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-45:HN | 1:VAL-45:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-45:HN | 1:THR-44:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-45:HN | 1:VAL-45:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-45:HN | 1:THR-44:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-45:HN | 1:THR-44:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL-45:HN | 1:MET-46:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA-25:HN | 1:PHE-26:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-81:HN | 1:GLU-77:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-81:HN | 1:LEU-79:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-81:HN | 1:VAL-80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-81:HN | 1:MET-81:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-81:HN | 1:VAL-80:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-81:HN | 1:MET-82:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-81:HN | 1:VAL-83:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE-61:HN | 1:ILE-61:HG11 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE-61:HN | 1:ALA-60:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE-61:HN | 1:ILE-61:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE-61:HN | 1:ILE-61:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE-61:HN | 1:ILE-62:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-58:HN | 1:THR-54:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-58:HN | 1:LEU-58:HG   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-58:HN | 1:LEU-58:HB*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU-58:HN | 1:ASP-59:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-18:HN | 1:MET-18:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-18:HN | 1:GLU-17:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-18:HN | 1:MET-18:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-18:HN | 1:MET-18:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-18:HN | 1:ILE-19:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET-18:HN | 1:GLU-17:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE-26:HN | 1:ALA-25:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE-26:HN | 1:PHE-26:HB*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE-26:HN | 1:PHE-26:HD*  | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE-26:HN | 1:ASP-27:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE-22:HN | 1:LEU-79:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |

|              |               |       |       |       |       |       |          |      |
|--------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:PHE_22:HN  | 1:ILE_19:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:GLU_21:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:GLU_21:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:PHE_22:HB*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:PHE_22:HE*  | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:PHE_22:HD*  | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:ILE_19:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:ILE_19:HG11 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:ILE_19:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:MET_18:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:MET_18:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:ILE_19:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:GLU_17:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:VAL_83:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:VAL_83:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:ARG+_84:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:VAL_83:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:ARG+_84:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:VAL_80:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:GLN_85:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HN | 1:VAL_83:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_77:HN  | 1:GLU_77:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_77:HN  | 1:GLU_77:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_77:HN  | 1:GLU_76:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_17:HN  | 1:GLU_17:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_17:HN  | 1:GLU_17:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_17:HN  | 1:GLU_17:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_17:HN  | 1:GLU_17:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:GLN_6:HG1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:VAL_80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:GLN_6:HB2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:GLN_6:HG2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:ASP_5:HB2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:GLN_6:HE22  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:GLN_7:HE22  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:ASP_5:HB1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:LYS+_87:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:LYS+_87:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:VAL_83:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:LYS+_87:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:LYS+_87:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:MET_86:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:MET_86:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:VAL_83:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_87:HN | 1:ASP_9:HN    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HN  | 1:VAL_80:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HN  | 1:VAL_80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HN  | 1:ALA_10:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HN  | 1:VAL_80:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_80:HN  | 1:LEU_79:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HN | 1:LEU_49:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HN | 1:LEU_58:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HN | 1:THR_44:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HN | 1:ARG+_47:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HN | 1:GLY_43:HA1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_47:HN | 1:MET_46:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:MET_28:HE*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:MET_28:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:MET_28:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:MET_28:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:MET_28:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:PHE_29:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:ASP_27:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HN  | 1:ALA_12:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HN  | 1:ARG+_11:HB* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HN  | 1:LEU_14:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HN  | 1:ARG+_11:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_9:HN   | 1:GLU_9:HB*   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_9:HN   | 1:GLU_9:HG1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_9:HN   | 1:GLU_9:HG2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_9:HN   | 1:GLN_7:HA    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:VAL_83:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:VAL_83:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:MET_82:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:LYS+_87:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:MET_86:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:MET_86:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:MET_86:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |



|              |               |       |       |       |       |       |          |      |
|--------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:MET_86:HN  | 1:ARG+_84:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_86:HN  | 1:GLN_85:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-59:HN | 1:ASP_-59:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-59:HN | 1:ASP_-59:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-59:HN | 1:LYS+_55:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HN  | 1:LEU_79:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HN  | 1:LEU_79:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HN  | 1:LEU_79:HG   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HN  | 1:PHE_75:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HN  | 1:MET_81:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_79:HN  | 1:GLU_-77:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HN  | 1:PHE_75:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HN  | 1:PHE_75:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HN  | 1:ASP_-74:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HN  | 1:GLU_-76:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HN  | 1:ILE_37:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HN  | 1:THR_44:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HN  | 1:THR_44:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HN  | 1:GLY_43:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:PHE_29:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:MET_28:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:ILE_37:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:ASP_-30:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:ASP_-30:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:PHE_29:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:PHE_29:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:LEU_58:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:ILE_62:HG11 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:ILE_62:HG12 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:ALA_60:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:GLU_-63:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HN  | 1:GLN_51:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HN  | 1:ASN_52:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HN  | 1:GLY_50:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:ILE_37:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:ASP_-30:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:MET_28:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:MET_28:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:MET_28:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:PHE_29:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:PHE_29:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:PHE_29:HD*  | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:LEU_14:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:LEU_14:HD2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:LEU_14:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:LEU_14:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:LEU_14:HG   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:ALA_12:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:PHE_13:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:PHE_13:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:LEU_14:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:LEU_14:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:ARG+_11:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:PHE_13:HD*  | 1.800 | 7.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:LEU_14:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:ARG+_11:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-63:HN | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-63:HN | 1:GLU_-63:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-63:HN | 1:GLU_-63:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-63:HN | 1:GLU_-64:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-27:HN | 1:LYS+_23:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HN  | 1:MET_82:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HN  | 1:MET_82:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HN  | 1:MET_82:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HN  | 1:MET_82:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HN  | 1:VAL_83:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_82:HN  | 1:VAL_83:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-27:HN | 1:LYS+_23:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-27:HN | 1:ALA_25:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-27:HN | 1:ASP_-27:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-27:HN | 1:ASP_-27:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HN  | 1:GLY_71:HA1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HN  | 1:SER_70:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HN  | 1:ASP_-68:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HN  | 1:GLY_69:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_70:HN  | 1:GLY_71:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |

|               |               |       |       |       |       |       |          |      |
|---------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:GLU_-56:HN  | 1:THR_54:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-56:HN  | 1:LYS+_55:HD1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-56:HN  | 1:GLU_-56:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-56:HN  | 1:GLU_-56:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-56:HN  | 1:GLU_-56:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HN   | 1:GLN_51:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HN   | 1:ASN_52:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HN   | 1:ASN_52:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HN   | 1:PRO_53:HD1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HN   | 1:PRO_53:HD2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HN   | 1:MET_46:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HN   | 1:MET_46:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HN   | 1:MET_46:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HN   | 1:GLN_85:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HN   | 1:GLN_85:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HN   | 1:GLN_85:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-76:HN  | 1:GLU_-76:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-76:HN  | 1:GLU_-76:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-76:HN  | 1:GLU_-76:HG* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HN   | 1:LEU_14:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HN   | 1:LEU_14:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HN   | 1:MET_18:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HN   | 1:SER_15:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN  | 1:LEU_79:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN  | 1:LEU_14:HD1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN  | 1:VAL_80:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN  | 1:ALA_10:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN  | 1:ARG+_11:HB* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:ALA_60:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-68:HN  | 1:ASP_-68:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-68:HN  | 1:ASP_-68:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-68:HN  | 1:GLY_69:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:ALA_60:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:GLU_-64:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:GLU_-64:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:GLU_-64:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:GLU_-64:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN  | 1:ILE_62:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-36:HN  | 1:ASP_-36:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-36:HN  | 1:GLY_35:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_4:HN    | 1:THR_4:HG2*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_4:HN    | 1:ASP_-5:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HN   | 1:THR_39:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HN   | 1:THR_39:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_54:HN   | 1:THR_54:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HN  | 1:ALA_31:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HN  | 1:ASP_-32:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HN  | 1:ASP_-32:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HN  | 1:GLY_33:HA*  | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HN  | 1:ALA_31:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HN  | 1:GLY_33:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HD22 | 1:ASN_52:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HD22 | 1:ASN_52:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HD21 | 1:ASN_52:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HD21 | 1:ASN_52:HB2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HD22 | 1:ASN_52:HD21 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HE22 | 1:GLN_51:HE21 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HE21  | 1:GLN_7:HG*   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HE22  | 1:GLN_7:HE21  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HE22  | 1:GLN_6:HB2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HE22  | 1:GLN_6:HG1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HE22  | 1:GLN_6:HG2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HE21  | 1:GLN_6:HG1   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HE21  | 1:GLN_6:HG2   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HE22  | 1:GLN_6:HE21  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HE21  | 1:THR_4:HG2*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HE22  | 1:THR_4:HG2*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HE22  | 1:ASP_-5:HB1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HE22 | 1:GLN_7:HG*   | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HE22 | 1:GLN_85:HG1  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HE22 | 1:GLN_85:HG2  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_85:HE22 | 1:GLN_85:HE21 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_33:HN   | 1:ALA_31:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_33:HN   | 1:ALA_31:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_69:HN   | 1:ASP_-66:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_69:HN   | 1:ASP_-68:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_69:HN   | 1:GLY_71:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |

|              |               |       |       |       |       |       |          |      |
|--------------|---------------|-------|-------|-------|-------|-------|----------|------|
| 1:GLY_34:HN  | 1:ASP_-30:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_34:HN  | 1:ASP_-32:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_34:HN  | 1:GLY_33:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_34:HN  | 1:GLY_35:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HN  | 1:ILE_62:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HN  | 1:THR_72:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HN  | 1:GLY_71:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HE | 1:VAL_80:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HE | 1:ARG+_84:HG1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HE | 1:ARG+_84:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_84:HE | 1:ARG+_84:HD* | 1.800 | 6.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-74:HN | 1:ILE_73:HG2* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-74:HN | 1:ASP_-74:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HN  | 1:VAL_65:HG1* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HN  | 1:VAL_65:HB   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALAC_90:HN | 1:ASP_-89:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALAC_90:HN | 1:ASP_-89:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALAC_90:HN | 1:ALAC_90:HB* | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_31:HN  | 1:ASP_-30:HB2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_31:HN  | 1:ALA_31:HB*  | 1.800 | 6.500 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_43:HN  | 1:THR_39:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLY_50:HN  | 1:LEU_49:HN   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-67:HN | 1:GLU_-67:HB1 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-67:HN | 1:GLU_-67:HG2 | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-67:HN | 1:ASP_-68:HN  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_4:HN   | 1:THR_4:HA    | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-5:HN  | 1:ASP_-5:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:GLN_6:HA    | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_6:HN   | 1:ASP_-5:HA   | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HN   | 1:GLN_7:HA    | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_7:HN   | 1:GLN_6:HA    | 2.300 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_8:HN   | 1:ALA_8:HA    | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_8:HN   | 1:GLN_7:HA    | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_8:HN   | 1:ASP_-5:HA   | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-9:HN  | 1:GLU_-9:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-9:HN  | 1:ALA_8:HA    | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-9:HN  | 1:GLN_6:HA    | 2.200 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HN  | 1:ALA_10:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HN  | 1:GLU_-9:HA   | 2.300 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_10:HN  | 1:GLN_7:HA    | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN | 1:ARG+_11:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN | 1:ALA_10:HA   | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ARG+_11:HN | 1:ALA_8:HA    | 2.800 | 4.300 | 4.300 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HN  | 1:ALA_12:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HN  | 1:ARG+_11:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_12:HN  | 1:GLU_-9:HA   | 2.800 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:PHE_13:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:ALA_12:HA   | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_13:HN  | 1:ALA_10:HA   | 2.800 | 4.300 | 4.300 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:LEU_14:HA   | 2.700 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:PHE_13:HA   | 2.100 | 2.700 | 2.700 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_14:HN  | 1:ARG+_11:HA  | 1.800 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_15:HN  | 1:LEU_14:HA   | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-15:HN | 1:GLU_-16:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-16:HN | 1:SER_15:HA   | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-17:HN | 1:GLU_-17:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-17:HN | 1:GLU_-16:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HN  | 1:MET_18:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_18:HN  | 1:GLU_-17:HA  | 2.900 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:ILE_19:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:MET_18:HA   | 2.900 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_19:HN  | 1:GLU_-16:HA  | 2.800 | 4.300 | 4.300 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_20:HN  | 1:ALA_20:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_20:HN  | 1:ILE_19:HA   | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_20:HN  | 1:GLU_-17:HA  | 2.800 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-21:HN | 1:GLU_-21:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-21:HN | 1:ALA_20:HA   | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-21:HN | 1:MET_18:HA   | 2.800 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:PHE_22:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:GLU_-21:HA  | 2.300 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_22:HN  | 1:ILE_19:HA   | 2.200 | 4.300 | 4.300 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HN | 1:LYS+_23:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HN | 1:PHE_22:HA   | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_23:HN | 1:ALA_20:HA   | 2.200 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_24:HN  | 1:ALA_24:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_24:HN  | 1:LYS+_23:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_24:HN  | 1:GLU_-21:HA  | 2.800 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_25:HN  | 1:ALA_25:HA   | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |

|              |              |       |       |       |       |       |          |      |
|--------------|--------------|-------|-------|-------|-------|-------|----------|------|
| 1:ALA_25:HN  | 1:ALA_24:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_25:HN  | 1:PHE_22:HA  | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_26:HN  | 1:PHE_26:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_26:HN  | 1:ALA_25:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_26:HN  | 1:LYS+_23:HA | 2.800 | 4.300 | 4.300 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-27:HN | 1:ASP_-27:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-27:HN | 1:PHE_26:HA  | 2.300 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:MET_28:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:ASP_-27:HA | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_28:HN  | 1:ALA_25:HA  | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:PHE_29:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:MET_28:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_29:HN  | 1:PHE_26:HA  | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:ASP_-30:HA | 2.700 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:PHE_29:HA  | 2.100 | 2.900 | 2.900 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-30:HN | 1:ASP_-27:HA | 1.800 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_31:HN  | 1:ALA_31:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_31:HN  | 1:ASP_-30:HA | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-32:HN | 1:ALA_31:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-36:HN | 1:ASP_-36:HA | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_37:HN  | 1:ASP_-36:HA | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:SER_38:HN  | 1:ILE_37:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HN  | 1:THR_39:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_39:HN  | 1:SER_38:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HN | 1:LYS+_40:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_40:HN | 1:THR_39:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-41:HN | 1:GLU_-41:HA | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HN  | 1:LEU_42:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HN  | 1:GLU_-41:HA | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_42:HN  | 1:THR_39:HA  | 2.800 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HN  | 1:THR_44:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_44:HN  | 1:THR_44:HA  | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_45:HN  | 1:VAL_45:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_45:HN  | 1:THR_44:HA  | 2.300 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_45:HN  | 1:LEU_42:HA  | 2.200 | 4.300 | 4.300 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HN  | 1:MET_46:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_46:HN  | 1:VAL_45:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HN  | 1:MET_48:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:MET_48:HN  | 1:VAL_45:HA  | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLN_51:HN  | 1:GLN_51:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HN  | 1:ASN_52:HA  | 2.700 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASN_52:HN  | 1:GLN_51:HA  | 2.100 | 2.700 | 2.700 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_54:HN  | 1:THR_54:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HN | 1:LYS+_55:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LYS+_55:HN | 1:THR_54:HA  | 2.900 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-56:HN | 1:GLU_-56:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-56:HN | 1:LYS+_55:HA | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-57:HN | 1:GLU_-57:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-57:HN | 1:GLU_-56:HA | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:LEU_58:HN  | 1:LYS+_55:HA | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-59:HN | 1:ASP_-59:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-59:HN | 1:LEU_58:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-59:HN | 1:GLU_-56:HA | 2.800 | 4.300 | 4.300 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_60:HN  | 1:ALA_60:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_60:HN  | 1:ASP_-59:HA | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ALA_60:HN  | 1:GLU_-57:HA | 2.800 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HN  | 1:ILE_61:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HN  | 1:ALA_60:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_61:HN  | 1:LEU_58:HA  | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:ILE_62:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:ILE_61:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_62:HN  | 1:ASP_-59:HA | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-63:HN | 1:GLU_-63:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-63:HN | 1:ILE_62:HA  | 3.100 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-63:HN | 1:ALA_60:HA  | 2.800 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN | 1:GLU_-64:HA | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN | 1:GLU_-63:HA | 2.900 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-64:HN | 1:ILE_61:HA  | 2.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HN  | 1:VAL_65:HA  | 2.700 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:VAL_65:HN  | 1:GLU_-64:HA | 2.100 | 2.700 | 2.700 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:GLU_-67:HN | 1:GLU_-67:HA | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:THR_72:HN  | 1:THR_72:HA  | 1.800 | 5.000 | 5.000 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HN  | 1:ILE_73:HA  | 2.700 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ILE_73:HN  | 1:THR_72:HA  | 2.100 | 2.700 | 2.700 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-74:HN | 1:ASP_-74:HA | 2.700 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:ASP_-74:HN | 1:ILE_73:HA  | 2.100 | 2.700 | 2.700 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HN  | 1:PHE_75:HA  | 2.600 | 3.100 | 3.100 | 10.00 | 10.00 | 1000.000 | 0.00 |
| 1:PHE_75:HN  | 1:ASP_-74:HA | 2.300 | 3.600 | 3.600 | 10.00 | 10.00 | 1000.000 | 0.00 |



|               |              |            |             |         |          |       |          |          |
|---------------|--------------|------------|-------------|---------|----------|-------|----------|----------|
| 1:GLU-76:HN   | 1:GLU-76:HA  | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLU-77:HN   | 1:GLU-77:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLU-77:HN   | 1:GLU-76:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:PHE-78:HN   | 1:PHE-78:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:PHE-78:HN   | 1:GLU-77:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:PHE-78:HN   | 1:PHE-75:HA  | 2.800      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:LEU-79:HN   | 1:PHE-78:HA  | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:LEU-79:HN   | 1:GLU-76:HA  | 1.800      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:VAL-80:HN   | 1:VAL-80:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:VAL-80:HN   | 1:LEU-79:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:VAL-80:HN   | 1:GLU-77:HA  | 2.800      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-81:HN   | 1:MET-81:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-81:HN   | 1:VAL-80:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-81:HN   | 1:PHE-78:HA  | 2.800      | 4.300       | 4.300   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-82:HN   | 1:MET-82:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-82:HN   | 1:MET-81:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-82:HN   | 1:LEU-79:HA  | 2.800      | 4.300       | 4.300   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:VAL-83:HN   | 1:VAL-83:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:VAL-83:HN   | 1:MET-82:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:VAL-83:HN   | 1:VAL-80:HA  | 2.800      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:ARG+84:HN   | 1:ARG+84:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:ARG+84:HN   | 1:VAL-83:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:ARG+84:HN   | 1:MET-81:HA  | 2.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLN-85:HN   | 1:GLN-85:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLN-85:HN   | 1:ARG+84:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLN-85:HN   | 1:MET-82:HA  | 2.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-86:HN   | 1:MET-86:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-86:HN   | 1:GLN-85:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-86:HN   | 1:VAL-83:HA  | 2.800      | 4.300       | 4.300   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:LYS+87:HN   | 1:LYS+87:HA  | 2.600      | 3.100       | 3.100   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:LYS+87:HN   | 1:MET-86:HA  | 3.100      | 3.600       | 3.600   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:LYS+87:HN   | 1:ARG+84:HA  | 2.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:ALAC-90:HN  | 1:ALAC-90:HA | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-33:HN   | 1:GLY-33:HA* | 1.800      | 6.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-34:HN   | 1:GLY-33:HA* | 1.800      | 6.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-34:HN   | 1:GLY-34:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-34:HN   | 1:GLY-34:HA2 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:ASP-36:HN   | 1:GLY-35:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-43:HN   | 1:GLY-43:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-43:HN   | 1:GLY-43:HA2 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:THR-44:HN   | 1:GLY-43:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:MET-46:HN   | 1:GLY-43:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-50:HN   | 1:LEU-49:HA  | 3.100      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-50:HN   | 1:GLY-50:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-50:HN   | 1:GLY-50:HA2 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLN-51:HN   | 1:GLY-50:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLN-51:HN   | 1:GLY-50:HA2 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-69:HN   | 1:GLY-69:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:GLY-69:HN   | 1:GLY-69:HA2 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:SER-70:HN   | 1:GLY-69:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:SER-70:HN   | 1:GLY-69:HA2 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:THR-72:HN   | 1:GLY-71:HA1 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| 1:THR-72:HN   | 1:GLY-71:HA2 | 1.800      | 5.000       | 5.000   | 10.00    | 10.00 | 1000.000 | 0.00     |
| !             |              |            |             |         |          |       |          |          |
| #NMR_dihedral |              |            |             |         |          |       |          |          |
| 1:ALAN-1:CA   | 1:ALAN-1:C   | 1:SER-2:N  | 1:SER-2:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:SER-2:CA    | 1:SER-2:C    | 1:MET-3:N  | 1:MET-3:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:MET-3:CA    | 1:MET-3:C    | 1:THR-4:N  | 1:THR-4:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:THR-4:CA    | 1:THR-4:C    | 1:ASP-5:N  | 1:ASP-5:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ASP-5:CA    | 1:ASP-5:C    | 1:GLN-6:N  | 1:GLN-6:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:GLN-6:CA    | 1:GLN-6:C    | 1:GLN-7:N  | 1:GLN-7:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:GLN-7:CA    | 1:GLN-7:C    | 1:ALA-8:N  | 1:ALA-8:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ALA-8:CA    | 1:ALA-8:C    | 1:GLU-9:N  | 1:GLU-9:CA  | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:GLU-9:CA    | 1:GLU-9:C    | 1:ALA-10:N | 1:ALA-10:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ALA-10:CA   | 1:ALA-10:C   | 1:ARG+11:N | 1:ARG+11:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ARG+11:CA   | 1:ARG+11:C   | 1:ALA-12:N | 1:ALA-12:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ALA-12:CA   | 1:ALA-12:C   | 1:PHE-13:N | 1:PHE-13:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:PHE-13:CA   | 1:PHE-13:C   | 1:LEU-14:N | 1:LEU-14:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:LEU-14:CA   | 1:LEU-14:C   | 1:SER-15:N | 1:SER-15:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:SER-15:CA   | 1:SER-15:C   | 1:GLU-16:N | 1:GLU-16:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:GLU-16:CA   | 1:GLU-16:C   | 1:GLU-17:N | 1:GLU-17:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:GLU-17:CA   | 1:GLU-17:C   | 1:MET-18:N | 1:MET-18:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:MET-18:CA   | 1:MET-18:C   | 1:ILE-19:N | 1:ILE-19:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ILE-19:CA   | 1:ILE-19:C   | 1:ALA-20:N | 1:ALA-20:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ALA-20:CA   | 1:ALA-20:C   | 1:GLU-21:N | 1:GLU-21:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:GLU-21:CA   | 1:GLU-21:C   | 1:PHE-22:N | 1:PHE-22:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:PHE-22:CA   | 1:PHE-22:C   | 1:LYS+23:N | 1:LYS+23:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:LYS+23:CA   | 1:LYS+23:C   | 1:ALA-24:N | 1:ALA-24:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ALA-24:CA   | 1:ALA-24:C   | 1:ALA-25:N | 1:ALA-25:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:ALA-25:CA   | 1:ALA-25:C   | 1:PHE-26:N | 1:PHE-26:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |
| 1:PHE-26:CA   | 1:PHE-26:C   | 1:ASP-27:N | 1:ASP-27:CA | 175.000 | -175.000 | 10.00 | 10.00    | 1000.000 |

|             |            |             |              |         |          |       |       |          |
|-------------|------------|-------------|--------------|---------|----------|-------|-------|----------|
| 1:ASP-27:CA | 1:ASP-27:C | 1:MET-28:N  | 1:MET-28:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:MET-28:CA | 1:MET-28:C | 1:PHE-29:N  | 1:PHE-29:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:PHE-29:CA | 1:PHE-29:C | 1:ASP-30:N  | 1:ASP-30:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-30:CA | 1:ASP-30:C | 1:ALA-31:N  | 1:ALA-31:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ALA-31:CA | 1:ALA-31:C | 1:ASP-32:N  | 1:ASP-32:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-32:CA | 1:ASP-32:C | 1:GLY-33:N  | 1:GLY-33:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLY-33:CA | 1:GLY-33:C | 1:GLY-34:N  | 1:GLY-34:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLY-34:CA | 1:GLY-34:C | 1:GLY-35:N  | 1:GLY-35:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLY-35:CA | 1:GLY-35:C | 1:ASP-36:N  | 1:ASP-36:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-36:CA | 1:ASP-36:C | 1:ILE-37:N  | 1:ILE-37:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ILE-37:CA | 1:ILE-37:C | 1:SER-38:N  | 1:SER-38:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:SER-38:CA | 1:SER-38:C | 1:THR-39:N  | 1:THR-39:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:THR-39:CA | 1:THR-39:C | 1:LYS-40:N  | 1:LYS-40:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:LYS-40:CA | 1:LYS-40:C | 1:GLU-41:N  | 1:GLU-41:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-41:CA | 1:GLU-41:C | 1:LEU-42:N  | 1:LEU-42:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:LEU-42:CA | 1:LEU-42:C | 1:GLY-43:N  | 1:GLY-43:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLY-43:CA | 1:GLY-43:C | 1:THR-44:N  | 1:THR-44:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:THR-44:CA | 1:THR-44:C | 1:VAL-45:N  | 1:VAL-45:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:VAL-45:CA | 1:VAL-45:C | 1:MET-46:N  | 1:MET-46:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:MET-46:CA | 1:MET-46:C | 1:ARG-47:N  | 1:ARG-47:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ARG-47:CA | 1:ARG-47:C | 1:MET-48:N  | 1:MET-48:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:MET-48:CA | 1:MET-48:C | 1:LEU-49:N  | 1:LEU-49:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:LEU-49:CA | 1:LEU-49:C | 1:GLY-50:N  | 1:GLY-50:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLY-50:CA | 1:GLY-50:C | 1:GLN-51:N  | 1:GLN-51:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLN-51:CA | 1:GLN-51:C | 1:ASN-52:N  | 1:ASN-52:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASN-52:CA | 1:ASN-52:C | 1:PRO-53:N  | 1:PRO-53:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:PRO-53:CA | 1:PRO-53:C | 1:THR-54:N  | 1:THR-54:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:THR-54:CA | 1:THR-54:C | 1:LYS-55:N  | 1:LYS-55:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:LYS-55:CA | 1:LYS-55:C | 1:GLU-56:N  | 1:GLU-56:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-56:CA | 1:GLU-56:C | 1:GLU-57:N  | 1:GLU-57:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-57:CA | 1:GLU-57:C | 1:LEU-58:N  | 1:LEU-58:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:LEU-58:CA | 1:LEU-58:C | 1:ASP-59:N  | 1:ASP-59:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-59:CA | 1:ASP-59:C | 1:ALA-60:N  | 1:ALA-60:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ALA-60:CA | 1:ALA-60:C | 1:ILE-61:N  | 1:ILE-61:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ILE-61:CA | 1:ILE-61:C | 1:ILE-62:N  | 1:ILE-62:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ILE-62:CA | 1:ILE-62:C | 1:GLU-63:N  | 1:GLU-63:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-63:CA | 1:GLU-63:C | 1:GLU-64:N  | 1:GLU-64:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-64:CA | 1:GLU-64:C | 1:VAL-65:N  | 1:VAL-65:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:VAL-65:CA | 1:VAL-65:C | 1:ASP-66:N  | 1:ASP-66:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-66:CA | 1:ASP-66:C | 1:GLU-67:N  | 1:GLU-67:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-67:CA | 1:GLU-67:C | 1:ASP-68:N  | 1:ASP-68:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-68:CA | 1:ASP-68:C | 1:GLY-69:N  | 1:GLY-69:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLY-69:CA | 1:GLY-69:C | 1:SER-70:N  | 1:SER-70:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:SER-70:CA | 1:SER-70:C | 1:GLY-71:N  | 1:GLY-71:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLY-71:CA | 1:GLY-71:C | 1:THR-72:N  | 1:THR-72:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:THR-72:CA | 1:THR-72:C | 1:ILE-73:N  | 1:ILE-73:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ILE-73:CA | 1:ILE-73:C | 1:ASP-74:N  | 1:ASP-74:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-74:CA | 1:ASP-74:C | 1:PHE-75:N  | 1:PHE-75:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:PHE-75:CA | 1:PHE-75:C | 1:GLU-76:N  | 1:GLU-76:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-76:CA | 1:GLU-76:C | 1:GLU-77:N  | 1:GLU-77:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-77:CA | 1:GLU-77:C | 1:PHE-78:N  | 1:PHE-78:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:PHE-78:CA | 1:PHE-78:C | 1:LEU-79:N  | 1:LEU-79:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:LEU-79:CA | 1:LEU-79:C | 1:VAL-80:N  | 1:VAL-80:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:VAL-80:CA | 1:VAL-80:C | 1:MET-81:N  | 1:MET-81:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:MET-81:CA | 1:MET-81:C | 1:MET-82:N  | 1:MET-82:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:MET-82:CA | 1:MET-82:C | 1:VAL-83:N  | 1:VAL-83:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:VAL-83:CA | 1:VAL-83:C | 1:ARG-84:N  | 1:ARG-84:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ARG-84:CA | 1:ARG-84:C | 1:GLN-85:N  | 1:GLN-85:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLN-85:CA | 1:GLN-85:C | 1:MET-86:N  | 1:MET-86:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:MET-86:CA | 1:MET-86:C | 1:LYS-87:N  | 1:LYS-87:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:LYS-87:CA | 1:LYS-87:C | 1:GLU-88:N  | 1:GLU-88:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:GLU-88:CA | 1:GLU-88:C | 1:ASP-89:N  | 1:ASP-89:CA  | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |
| 1:ASP-89:CA | 1:ASP-89:C | 1:ALAC-90:N | 1:ALAC-90:CA | 175.000 | -175.000 | 10.00 | 10.00 | 1000.000 |

## Calculation parameters

This is the parameter file used by the Biosym DGII program to generate the 64 structures.

```
NAME_0="BIOSYM DGII NMR_project=ntnc_5 mol=NTNC_5 Wed Jan 5 11:53:44 1994"
export NAME_0 # <= 80 char description of system
BASE_0=ntnc_5; export BASE_0 # base name for all associated files
NSTR_0=40; export NSTR_0 # number of structures to try to make
ARCH_0=iris; export ARCH_0 # architecture of computer to use
NICE_0=19; export NICE_0 # niceness of calculations
LOGF_0=mail; export LOGF_0 # log file (mail ==> mailed to user)
TRAN_1=step1; export TRAN_1 # base of transcript file for step1
VERB_1=silent; export VERB_1 # verbosity of step1 transcript
RLIB_1=stereo.lib; export RLIB_1 # residue library in DISGEO format
LLIB_1=aalink.lib; export LLIB_1 # link library in DISGEO format
ALIB_1=nradii.lib; export ALIB_1 # atom radii library in DISGEO format
OMEG_1=10; # omega wobble in degrees
TRAN_2=step2; export TRAN_2 # base of transcript file for step2
VERB_2=report; export VERB_2 # verbosity of step2 transcript
PREC_2=0.0001; export PREC_2 # precision of input bounds for step2
TOLN_2=0.1; export TOLN_2 # tolerance for violations for step2
TRI1_2=yes; export TRI1_2 # control for triangle smoothing
PLAN_2=group; export PLAN_2 # strategy for tetrahedron smoothing
CRIT_2=0.1; export CRIT_2 # convergence criterion for tetrahedron
PASS_2=1; export PASS_2 # maximum number of passes in tetrahedron
TRAN_3=step3; export TRAN_3 # base of transcript for step3
VERB_3=report; export VERB_3 # verbosity of step3 calculations
METH_3=prospective; export METH_3 # method to use for selection of distances
ALPH_3=uniform; export ALPH_3 # probability distribution for selection
EDIM_3=4; export EDIM_3 # dimension in which to embed coordinates
PRES_3=no; export PRES_3 # preserve the radius of gyration
IMAX_3=200; export IMAX_3 # iteration limit for eigencalculation
ECVG_3=0.0001; export ECVG_3 # convergence criteria for eigencalculation
CRIT_3=0.001; export CRIT_3 # criterion of inverse calculation
GTSV_3=20; # saved value of Guttman transformations
GUTT_3=20; export GUTT_3 # number of Guttman transformations
LCGI_3=100; export LCGI_3 # no. iterations for linear conjugate
gradient
LCGK_3=0.001; export LCGK_3 # conv. criteria for linear conjugate
gradient
LSWF_3=range_inv; export LSWF_3 # type of weights for least-squares fit
CMPI_3=yes; export CMPI_3 # Calculate MP Inverse? during R & A
majorize
RMDM_3=yes; export RMDM_3 # remove old distance matrix files
TRAN_4=step4; export TRAN_4 # base of transcript for step4
WCHI_4=0.1; export WCHI_4 # weight to give chirality error
WDIM_4=0.2; export WDIM_4 # weight to give dimensionality error
PROJ_4=0.3; export PROJ_4 # factor by which to project start
UPWT_4=1; export UPWT_4 # upper weight limit for cook and cool
KCSV_4=2800; # saved initial energy in kcal / mole
KCAL_4=2800; export KCAL_4 # initial energy in kcal / mole
XRAD_4=1; export XRAD_4 # extra radius (-x opt for cook and cool)
TMAX_4=200; export TMAX_4 # maximum temperature in degrees K
TINI_4=1; export TINI_4 # initial temperature in degrees K
HEAT_4=2; export HEAT_4 # max. fractional temp. incr. per step
STEP_4=2e-13; export STEP_4 # step size for simulation in sec.
MASS_4=1000; export MASS_4 # mass of all atoms in Daltons
FAIL_4=0.01; export FAIL_4 # annealing a failure if error exceeds
KLIM_4=20000; export KLIM_4 # maximum number of simulation steps
KOSV_4=100; # Saved value of KOUT
KOUT_4=100; export KOUT_4 # one line of output every KOUT steps
LLIM_4=250; export LLIM_4 # minimization iteration limit
LOUT_4=10; export LOUT_4 # one line of output every LOUT iter's
GRAD_4=0.0001; export GRAD_4 # min. quits if RMS gradient exceeds
PRSV_4=200; # saved value of preview SA
PREV_4=0; export PREV_4 # preview SA for this number steps
LBWT_4=10; export LBWT_4 # lower bound weight
CTWT_4=1; export CTWT_4 # contact weight
EFXF_4=sparse; export EFXF_4 # error function form
```